

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representation of
The original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

THIS PAGE BLANK (USPTO)

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 39/39, 9/16		A1	(11) International Publication Number: WO 99/27961 (43) International Publication Date: 10 June 1999 (10.06.99)
<p>(21) International Application Number: PCT/US98/25563</p> <p>(22) International Filing Date: 2 December 1998 (02.12.98)</p> <p>(30) Priority Data: 60/067,146 2 December 1997 (02.12.97) US 60/082,686 22 April 1998 (22.04.98) US</p> <p>(71) Applicant: POWDERJECT VACCINES, INC. [US/US]; Suite C, 585 Science Drive, Madison, WI 53711 (US).</p> <p>(72) Inventors: SARPHIE, David; 78 Hailey Road, Witney OX8 5HF (GB). SWAIN, William, F.; 4922 Marathon Drive, Madison, WI 53562 (US). WIDERA, Georg, J.; 3665 Camito Cielo Del Mar, San Diego, CA 92130 (US). DRAPE, Robert, J.; 1517 McKenna Boulevard #4, Madison, WI 53711 (US). CHEN, Dexiang; 9 Scranton Court, Madison, WI 53719 (US).</p> <p>(74) Agents: McCracken, Thomas, P. et al.; Robins & Associates, Suite 200, 90 Middlefield Road, Menlo Park, CA 94025 (US).</p>		<p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW. ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>	

(54) Title: TRANSDERMAL DELIVERY OF PARTICULATE VACCINE COMPOSITIONS

(57) Abstract

A method for enhancing the immune response to a selected antigen is disclosed. The method entails delivering a particulate adjuvant composition transdermally, preferably using a needleless syringe system. Also described are methods for forming crystalline particles from pharmaceutical compositions and then delivering the same to a subject. The crystallized compositions are particularly suitable for transdermal vaccine delivery using a needleless syringe system.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

5 TRANSDERMAL DELIVERY OF PARTICULATE VACCINE COMPOSITIONS

Technical Field

10 The invention relates to particulate compositions. More particularly, the invention relates to methods of delivering particulate formulations, as well as methods for forming crystalline particles from pharmaceutical compositions and then delivering the same to a subject. The 15 particulate compositions are particularly suitable for transdermal vaccine delivery using a needleless syringe system.

Background of the Invention

20 The ability to deliver agents into and through skin surfaces (transdermal delivery) provides many advantages over parenteral delivery techniques. In particular, transdermal delivery provides a safe, 25 convenient and noninvasive alternative to traditional administration systems, conveniently avoiding the major problems associated with parenteral delivery, e.g., needle pain, the risk of introducing infection to treated individuals, the risk of contamination or infection of health care workers caused by accidental 30 needle-sticks and the disposal of used needles. In addition, such delivery affords a high degree of control over blood concentrations of administered drugs.

35 Recently, a novel transdermal drug delivery system that entails the use of a needleless syringe to fire solid drug-containing particles in controlled

doses into and through intact skin has been described. In particular, commonly owned U.S. Patent No. 5,630,796 to Bellhouse et al., describes a needleless syringe that delivers pharmaceutical particles 5 entrained in a supersonic gas flow. The needleless syringe (also referred to as "the PowderJect needleless syringe device") is used for transdermal delivery of powdered drug compounds and compositions, for delivery of genetic material into living cells 10 (e.g., gene therapy) and for the delivery of biopharmaceuticals to skin, muscle, blood or lymph. The needleless syringe can also be used in conjunction with surgery to deliver drugs and biologics to organ surfaces, solid tumors and/or to surgical cavities 15 (e.g., tumor beds or cavities after tumor resection). Pharmaceutical agents that can be suitably prepared in a substantially solid, particulate form can be safely and easily delivered using such a device.

One particular needleless syringe generally 20 comprises an elongate tubular nozzle having a rupturable membrane initially closing the passage through the nozzle and arranged substantially adjacent to the upstream end of the nozzle. Particles of a therapeutic agent to be delivered are disposed 25 adjacent to the rupturable membrane and are delivered using an energizing means which applies a gaseous pressure to the upstream side of the membrane sufficient to burst the membrane and produce a supersonic gas flow (containing the pharmaceutical 30 particles) through the nozzle for delivery from the downstream end thereof. The particles can thus be delivered from the needleless syringe at delivery velocities of between Mach 1 and Mach 8 which are readily obtainable upon the bursting of the rupturable 35 membrane.

Another needleless syringe configuration generally includes the same elements as described above, except that instead of having the pharmaceutical particles entrained within a supersonic gas flow, the downstream end of the nozzle is provided with a bistable diaphragm which is moveable between a resting "inverted" position (in which the diaphragm presents a concavity on the downstream face to contain the pharmaceutical particles) and an active "everted" position (in which the diaphragm is outwardly convex on the downstream face as a result of a supersonic shockwave having been applied to the upstream face of the diaphragm). In this manner, the pharmaceutical particles contained within the concavity of the diaphragm are expelled at a supersonic initial velocity from the device for transdermal delivery thereof to a targeted skin or mucosal surface.

Transdermal delivery using the above-described needleless syringe configurations is carried out with particles having an approximate size that generally ranges between 0.1 and 250 μm . Particles larger than about 250 μm can also be delivered from the device, with the upper limitation being the point at which the size of the particles would cause untoward damage to the skin cells. The actual distance which the delivered particles will penetrate depends upon particle size (e.g., the nominal particle diameter assuming a roughly spherical particle geometry), particle density, the initial velocity at which the particle impacts the skin surface, and the density and kinematic viscosity of the skin. Target particle densities for use in needleless injection generally range between about 0.1 and 25 g/cm^3 , and injection velocities generally range between about 200 and 3,000 m/sec .

A particularly unique feature of the
needleless syringe is the ability to closely control
the depth of penetration of delivered particles,
thereby allowing for targeted administration of
5 pharmaceuticals to various sites. For example,
particle characteristics and/or device operating
parameters can be selected to provide for varying
penetration depths for, e.g., intradermal or
subcutaneous delivery. One approach entails the
10 selection of particle size, particle density and
initial velocity to provide a momentum density (e.g.,
particle momentum divided by particle frontal area) of
between about 2 and 10 kg/sec/m, and more preferably
between about 4 and 7 kg/sec/m. Such control over
15 momentum density allows for precisely controlled,
tissue-selective delivery of the pharmaceutical
particles.

The above-described systems provide a unique
means for delivering vaccine antigens into or across
20 skin or tissue. However, many antigens require the
use of immunological adjuvants in order to increase
antigenic potency. Immunological adjuvants act to
augment cell-mediated and humoral immune responses.
Such adjuvants include depot adjuvants, compounds
25 which adsorb and/or precipitate administered antigens
and which serve to retain the antigen at the injection
site. Typical depot adjuvants include aluminum
compounds and water-in-oil emulsions.

Depot adjuvants, although increasing
30 antigenicity, often provoke severe persistent local
reactions, such as granulomas, abscesses and scarring,
when injected subcutaneously or intramuscularly.
Other adjuvants, such as lipopolysaccharides and
35 muramyl dipeptides, can elicit pyrogenic responses
upon injection and/or Reiter's symptoms (influenza-
like symptoms, generalized joint discomfort and

sometimes anterior uveitis, arthritis and urethritis). Accordingly, there is a continued need for effective and safe delivery methods of adjuvants for enhancing immune responses to a given antigen.

5

Summary of the Invention

The present invention provides unique adjuvant and vaccine compositions as well as a unique 10 system for delivering particulate pharmaceutical compositions, including vaccines and other therapeutic agents. Novel methods for making particulate pharmaceutical compositions are also provided.

In one embodiment, then, a method is 15 provided for enhancing the immunogenicity of a selected antigen. The method comprises:

(a) administering an effective amount of the antigen to a vertebrate subject; and

(b) administering an amount of a particulate 20 adjuvant composition sufficient to enhance the immunogenicity of the antigen, wherein the adjuvant is delivered into or across skin or tissue of the vertebrate subject and further wherein the administering is carried out using a transdermal 25 delivery technique.

The antigen and adjuvant may be present in the same or different compositions and may be administered to the same or different sites in the vertebrate subject. Furthermore, the antigen may be 30 administered prior or subsequent to, or concurrently with the adjuvant composition.

In particularly preferred embodiments, the adjuvant and/or antigen are administered using a needleless syringe delivery device.

35 In another embodiment, the subject invention is directed to a method for eliciting an immune

response in a vertebrate subject. The method comprises transdermally delivering a particulate vaccine composition into or across skin or tissue of the vertebrate subject. The particulate vaccine composition comprises:

- 5 (a) an effective amount of a selected antigen; and
- (b) an amount of an adjuvant sufficient to enhance the immunogenicity of the antigen.

10 In yet another embodiment, the invention is directed to a particulate adjuvant composition suitable for delivery into or across skin or tissue of a vertebrate subject using a transdermal delivery technique. The particulate adjuvant composition can be used to elicit a physiological effect in a vertebrate subject by administering an amount of the particulate adjuvant composition into or across skin or tissue of the vertebrate subject sufficient to bring about the physiological effect.

15 20 25 30 35 In another embodiment, a method is provided for converting conventional pharmaceutical formulations into crystallized particles that are optimally suited for transdermal delivery using a needleless syringe. Thus, in one aspect of the invention, a liquid pharmaceutical formulation (e.g., either in aqueous form, or a reconstituted lyophilized product), is combined with a suitable excipient, for example a sugar, and then dried to provide a crystalline composition. The excipient is selected to provide for sufficient rigidity, structure, and density in the resulting crystalline product. The crystalline composition, now having a sufficient density, can be used directly in a needleless syringe delivery technique, or can be further processed to provide a more finely divided and/or uniform crystalline composition.

In a further embodiment of the invention, a crystalline pharmaceutical composition is delivered to a subject in order to bring about a desired treatment. In one particular aspect, a crystalline vaccine 5 composition is delivered to a subject via needleless injection in order to provide for a biological response in the subject. In a preferred embodiment, the crystalline vaccine composition is delivered to the subject to elicit an antigen-specific immune 10 response in the subject.

In yet another embodiment of the invention, a crystalline pharmaceutical composition is provided. The crystalline pharmaceutical has sufficient particle structure, rigidity and/or density characteristics 15 which renders it suitable for delivery into and/or through skin or mucosal tissue using a needleless syringe system. The crystalline pharmaceutical composition of the present invention can be made using the methods of the invention, and thus includes 20 vaccine compositions.

These and other embodiments of the invention will readily occur to those of ordinary skill in the art in view of the disclosure herein.

25 Brief Description of the Drawings

Figures 1A and 1B depict the results of Example 1 where the effect of particle size on IgG antibody response was assessed in particulate vaccine formulations.

30 Figures 2-4 depict ELISA results from sera obtained from mice immunized with a crystalline Hib conjugate vaccine composition delivered using a needleless syringe. In Figure 2, the PRP-CRM197 conjugate was used as the capture phase, in Figure 3, diphtheria toxoid was used as the capture phase, and 35

in Figure 4, a PRP-HSA conjugate was used as the capture phase.

Figure 5 depicts the IgG antibody response in subjects receiving descending doses of the Hib conjugate vaccine compositions in either particulate or liquid form.

Figures 6A and 6B depict the duration of immunity provided by the Hib conjugate vaccine compositions in either particulate or liquid form.

Figure 7 depicts antibody responses to an inactivated influenza virus vaccine composition delivered in either particulate or liquid form. The data represent geometric mean serum IgG titers from pooled sera.

Figures 8A and 8B depict the results of an influenza virus challenge study in subjects immunized with an inactivated influenza virus vaccine composition delivered in either particulate or liquid form. Subjects in Figure 8A received 25 μ g of inactivated virus, while subjects in Figure 8B received 5 μ g of inactivated virus. The data represent weight loss as mean percentage of initial body weight.

Figure 9 depicts the results of an influenza virus challenge study in subjects immunized with an inactivated influenza virus vaccine composition adjuvanted with an Alum adjuvant and delivered in either particulate or liquid form. The data represent weight loss as the mean percentage of initial body weight from eight animals.

Figure 10 depicts the results of an influenza virus challenge study in subjects immunized with an inactivated influenza virus vaccine composition adjuvanted with a PCPP adjuvant and delivered in either particulate or liquid form. The

data represent weight loss as the mean percentage of initial body weight from eight animals.

Figures 11A and 11B depict the results of influenza virus challenge studies in subjects immunized with an inactivated influenza virus vaccine composition adjuvanted with a CpG adjuvant and delivered in either particulate (Figure 11A) or liquid (Figure 11B) form. The data represent weight loss as the mean percentage of initial body weight from eight animals.

Figure 12 depicts the results of an influenza virus challenge study in subjects immunized with an inactivated influenza virus vaccine composition adjuvanted with a MPL adjuvant and delivered in either particulate or liquid form. The data represent weight loss as the mean percentage of initial body weight from eight animals.

Detailed Description of the Preferred Embodiments

Before describing the present invention in detail, it is to be understood that this invention is not limited to particular pharmaceutical formulations or process parameters as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting.

It must be noted that, as used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "a pharmaceutical agent" includes a mixture of two or more pharmaceutical agents, reference to "an antigen" includes a mixture of two or more antigens, reference to "an excipient"

includes mixtures of two or more excipients, and the like.

A. Definitions

5 Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although a number of methods and materials similar or equivalent to those described herein can be used in the practice 10 of the present invention, the preferred materials and methods are described herein.

15 In describing the present invention, the following terms will be employed, and are intended to be defined as indicated below.

20 By "transdermal delivery" is meant delivery of particles to target tissue to provide a local, regional or systemic response. This contrasts with the direct introduction of substances across cell membranes into living cells and tissue which is designed to operate on an intracellular level. Preferably, the particle size of the administered substance is larger than the cells present in the targeted tissue. Generally, for mammalian cells, 25 particles larger than 10 μM will achieve this desired effect. Suitable size ranges for particles are discussed further below.

30 Thus, the term "transdermal" delivery intends intradermal (e.g., into the dermis or epidermis), transdermal (e.g., "percutaneous") and transmucosal administration, i.e., delivery by passage of an agent into or through skin or mucosal tissue. See, e.g., *Transdermal Drug Delivery: Developmental Issues and Research Initiatives*, Hadgraft and Guy (eds.), Marcel Dekker, Inc., (1989); *Controlled Drug Delivery: Fundamentals and Applications*, Robinson and

Lee (eds.), Marcel Dekker Inc., (1987); and *Transdermal Delivery of Drugs*, Vols. 1-3, Kydonieus and Berner (eds.), CRC Press, (1987).

By "needleless syringe" is meant an 5 instrument which delivers a particulate composition transdermally, without a conventional needle that pierces the skin. Needleless syringes for use with the present invention are discussed throughout this document.

10 As used herein, the term "pharmaceutical agent" intends any compound or composition of matter which, when administered to an organism (human or animal) induces a desired pharmacologic and/or physiologic effect by local and/or systemic action.

15 The term therefore encompasses those compounds or chemicals traditionally regarded as drugs and vaccines, as well as biopharmaceuticals including molecules such as peptides, hormones, nucleic acids, gene constructs and the like.

20 By "antigen" is meant a molecule which contains one or more epitopes that will stimulate a host's immune system to make a cellular antigen-specific immune response, or a humoral antibody response. Thus, antigens include proteins, 25 polypeptides, antigenic protein fragments, oligosaccharides, polysaccharides, and the like. Furthermore, the antigen can be derived from any known virus, bacterium, parasite, plants, protozoans, or fungus, and can be a whole organism. The term also 30 includes tumor antigens. Similarly, an oligonucleotide or polynucleotide which expresses an antigen, such as in DNA immunization applications, is also included in the definition of antigen. Synthetic antigens are also included, for example, polyepitopes, 35 flanking epitopes, and other recombinant or synthetically derived antigens (Bergmann et al. (1993)

Eur. J. Immunol. 23:2777-2781; Bergmann et al. (1996)
J. Immunol. 157:3242-3249; Suhrbier, A. (1997)
Immunol. and Cell Biol. 75:402-408; Gardner et al.
(1998) 12th World AIDS Conference, Geneva,
5 Switzerland, June 28-July 3, 1998).

The term "vaccine composition" intends any pharmaceutical composition containing an antigen, which composition can be used to prevent or treat a disease or condition in a subject. The term thus 10 encompasses both subunit vaccines, i.e., vaccine compositions containing antigens which are separate and discrete from a whole organism with which the antigen is associated in nature, as well as compositions containing whole killed, attenuated or 15 inactivated bacteria, viruses, parasites or other microbes.

Viral vaccine compositions used herein include, but are not limited to, those containing, or derived from, members of the families Picornaviridae 20 (e.g., polioviruses, etc.); Caliciviridae; Togaviridae (e.g., rubella virus, dengue virus, etc.); Flaviviridae; Coronaviridae; Reoviridae; Birnaviridae; Rhabdoviridae (e.g., rabies virus, etc.); Filoviridae; Paramyxoviridae (e.g., mumps virus, 25 measles virus, respiratory syncytial virus, etc.); Orthomyxoviridae (e.g., influenza virus types A, B and C, etc.); Bunyaviridae; Arenaviridae; Retroviridae (e.g., HTLV-I; HTLV-II; HIV-1; and HIV-2); simian immunodeficiency virus (SIV) among others. 30 Additionally, viral antigens may be derived from papillomavirus (e.g., HPV); a herpesvirus; a hepatitis virus, e.g., hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), the delta hepatitis virus (HDV), hepatitis E virus (HEV) and 35 hepatitis G virus (HGV); and the tick-borne encephalitis viruses. See, e.g. Virology, 3rd Edition

(W.K. Joklik ed. 1988); *Fundamental Virology*, 2nd Edition (B.N. Fields and D.M. Knipe, eds. 1991), for a description of these and other viruses. Bacterial vaccine compositions used herein include, but are not limited to, those containing or derived from organisms that cause diphtheria, cholera, tuberculosis, tetanus, pertussis, meningitis, and other pathogenic states, including, *Meningococcus A, B and C*, *Hemophilus influenza* type B (HIB), and *Helicobacter pylori*.

10 Examples of anti-parasitic vaccine compositions include those derived from organisms causing malaria and Lyme disease.

A composition which contains a selected antigen along with an adjuvant, or a vaccine composition which is coadministered with the subject adjuvant, displays "enhanced immunogenicity" when it possesses a greater capacity to elicit an immune response than the immune response elicited by an equivalent amount of the antigen administered without the adjuvant. Thus, a vaccine composition may display "enhanced immunogenicity" because the antigen is more strongly immunogenic or because a lower dose or fewer doses of antigen are necessary to achieve an immune response in the subject to which the antigen is administered. Such enhanced immunogenicity can be determined by administering the adjuvant composition and antigen controls to animals and comparing antibody titers and/or cellular-mediated immunity against the two using standard assays such as radioimmunoassay, ELISAs, CTL assays, and the like, well known in the art. For purposes of the present invention, an "effective amount" of an adjuvant will be that amount which enhances an immunological response to a coadministered antigen such that the antigen displays enhanced immunogenicity as described above.

Similarly, an "effective amount" of an antigen is an amount which will stimulate an immune response in the subject to which the antigen is administered. The immune response may be a humoral, 5 cell-mediated and/or protective immune response.

As used herein, the term "coadministered" such as when an adjuvant is coadministered with a vaccine antigen, intends either the simultaneous or concurrent administration of adjuvant and antigen, 10 e.g., when the two are present in the same composition or administered in separate compositions at nearly the same time but at different sites, as well as the delivery of adjuvant and antigen in separate compositions at different times. For example, the 15 adjuvant composition may be delivered prior to or subsequent to delivery of the antigen at the same or a different site. The timing between adjuvant and antigen deliveries can range from about several minutes apart, to several hours apart, to several days apart. Furthermore, although the adjuvant composition 20 is delivered to the skin using transdermal delivery methods such as a needleless syringe, the vaccine composition may be delivered using conventional delivery techniques, such as by conventional syringes 25 and conventional vaccine guns.

As used herein, the term "treatment" includes any of following: the prevention of infection or reinfection; the reduction or elimination of symptoms; and the reduction or complete elimination of 30 a pathogen. Treatment may be effected prophylactically (prior to infection) or therapeutically (following infection).

By "vertebrate subject" is meant any member of the subphylum cordata, particularly mammals, 35 including, without limitation, humans and other primates. The term does not denote a particular age.

Thus, both adult and newborn individuals are intended to be covered.

Pharmaceutical agents, alone or in combination with other drugs or agents, are typically 5 prepared as pharmaceutical compositions which can contain one or more added materials such as carriers, vehicles, and/or excipients. "Carriers," "vehicles" and "excipients" generally refer to substantially inert materials which are nontoxic and do not interact 10 with other components of the composition in a deleterious manner. These materials can be used to increase the amount of solids in particulate pharmaceutical compositions, such as those prepared using spray-drying or lyophilization techniques.

15 Examples of normally employed "excipients" or "carriers" include pharmaceutical grades of dextrose, sucrose, lactose, trehalose, mannitol, sorbitol, inositol, dextran, starch, cellulose, sodium or calcium phosphates, calcium sulfate, citric or 20 tartaric acids (and pharmaceutically acceptable salts thereof), glycine, high molecular weight polyethylene glycols (PEG), and combinations thereof. Exemplary excipients that serve as stabilizers include commonly available cryoprotectants and antioxidants.

25

B. General Methods

The invention provides for delivery of particulate pharmaceutical compositions, particularly 30 vaccine compositions. These particulate compositions can be delivered to a subject using a transdermal needleless syringe delivery device. The ability to transdermally administer vaccine compositions in particulate (powder) form to tissue such as the skin provides a significant improvement over prior 35 vaccination methods which generally rely on conventional needle and syringe injection techniques.

In this regard, almost all current vaccines are administered by intramuscular injection. However, injected vaccines need to get to local draining lymph nodes in order to initiate an immune response. A 5 large portion of a vaccine composition injected intramuscularly will rapidly diffuse into surrounding tissue and circulation, thus becoming lost or diluted. In contrast, when vaccine compositions are delivered transdermally, e.g., to skin, such losses will not 10 occur. This is because the top layers of skin have stronger antigen retaining ability due to poor vascularity. Particulate vaccine compositions are also better retained in the skin because of a slower dissolving process. The cellular component in the 15 skin may also contribute to improved vaccine performance following transdermal administration. This is because there is a dense network of Langerhans cells in the epidermal layer and dendritic cells in the cutaneous layer of skin. These cells are 20 important in the initiation and maintenance of an immune response. By delivering vaccine compositions in the proximity of these immune cells, it is feasible to achieve a stronger immune response than by conventional intramuscular injection. These immune 25 cells may also pick up the vaccine and migrate to the local draining lymph node, thereby initiating an immune response.

Transdermal administration of particulate compositions to skin or mucosal tissue also improves 30 the safety and efficacy of commonly used immunomodulators such as adjuvants. Immunomodulators are often important components of vaccines and immune therapeutics. Immunomodulators have many functions including, for example, immune enhancement, 35 immunosuppression, and immune modulation. Immune enhancement improves the efficacy of a vaccine or

immunotherapeutics. Immune enhancement also enables the immune system to respond to smaller doses of vaccine compositions. For example, the aluminum (Alum) adjuvant immunomodulator is used to formulate diphtheria and tetanus toxoid vaccines to improve their immunogenicity. Some immunomodulators that have immunosuppressive properties are also useful in treating certain diseases, such as autoimmune diseases and organ transplantation. Immunomodulators can also direct the immune systems to generate either a Th1- or Th2-type of response, or to switch one type of established response to another type. This immune modulating property is very important in immunotherapy. For example, subjects having an immune system biased toward a Th2-type response tend to have allergies. Immunomodulators which can help promote a Th1-type response are thus useful in immune therapy to desensitize those individuals.

As with conventional vaccine compositions, immunomodulators are typically administered by intramuscular injection. One of the problems with this injection method is the toxicity of immunomodulators after they have reached systemic circulation. It is for this reason that many immunomodulators can not be used in humans. Intramuscular injection also requires a high dose of immunomodulators (relative to transdermally delivered immunomodulators) in order to be effective since a large portion of injected material will rapidly diffuse from the injection site, generally entering into the circulation. In this regard, an adjuvant may need to exert its activities at the injection site or in the local draining lymph node(s) to enhance vaccine performance.

Transdermal delivery of immunomodulators to skin or mucosal tissue in accordance with the present

invention is advantageous for the following reasons. First, the skin and mucosa are very potent parts of the immune system. As discussed above, there is a dense network of immune cells in the various layers of skin (e.g., Langerhans cells in the epidermal layer and dendritic cells in the cutaneous layer). Mucosal epithelium cells contain large number of intraepithelial dendritic cells. These cells are important in the initiation and maintenance of an immune response, making them prime targets for immunomodulation. By delivering immunomodulators in close proximity to these cells, and avoiding rapid loss by diffusion, it is feasible to achieve a stronger immunomodulation effect than by intramuscular injection. The effective dose of immunomodulators can also be significantly reduced. A lower dose helps reduce toxicity associated with many immunomodulators.

Accordingly, in one embodiment, the invention entails a procedure for forming crystalline particles (suitable for transdermal delivery) from conventional pharmaceutical preparations. Although the methods of the invention are broadly applicable to any pharmaceutical composition, the invention is exemplified herein with particular reference to methods which use liquid (aqueous) or reconstituted dried vaccine compositions as starting materials.

One common method of preparing and storing vaccine pharmaceuticals involves lyophilization (freeze-drying). Lyophilization relates to a technique for removing moisture from a material and involves rapid freezing at a very low temperature, followed by rapid dehydration by sublimation in a high vacuum. This technique typically yields low-density porous particles having an open matrix structure. Such particles are chemically stable, but are rapidly

reconstituted (disintegrated and/or brought into solution) when introduced into an aqueous environment.

Another method of preparing and storing vaccine compositions from delicate or heat-sensitive biomolecules is spray-drying. Spray-drying relates to the atomization of a solution of one or more solids using a nozzle, spinning disk or other device, followed by evaporation of the solvent from the droplets. More particularly, spray-drying involves combining a highly dispersed liquid pharmaceutical preparation (e.g., a solution, slurry, emulsion or the like) with a suitable volume of hot air to produce evaporation and drying of the liquid droplets. Spray-dried pharmaceuticals are generally characterized as homogenous spherical particles that are frequently hollow. Such particles have low density and exhibit a rapid rate of solution.

In one method of the invention, a liquid composition, (an aqueous or a reconstituted spray-dried or lyophilized composition) is converted into a dry, crystalline powder suitable for delivery into and/or through skin or mucosal tissues. The liquid composition can be combined with a suitable carrier or excipient which provides for enhanced crystal formation, particle structure, rigidity and/or density characteristics. Preferred carriers or excipients include pharmaceutical-grade sugars and the like including, for example, trehalose. The composition is then allowed to dry under suitable evaporative conditions, resulting in a crystallized composition. The crystals can then be removed from the drying surface or container, and lightly broken up, for example, using mortar and pestle. The resulting crystalline powder can then be loaded into suitable delivery cassettes for delivery to a subject using a needleless syringe.

Although not limiting in the present invention, the above-described method can be used to obtain crystalline particles having a size ranging from about 0.1 to about 250 μm , preferably about 10 to 5 about 250 μm and a particle density ranging from about 0.1 to about 25 g/cm³. These crystalline particles can be used in the treatment or prevention of a variety of diseases.

10 In another embodiment, the invention pertains to delivery of particulate compositions, particularly adjuvant and vaccine compositions. The adjuvant and vaccine compositions may be in crystalline form, as described above, or may be delivered in an uncryallized, particulate state.

15 Antigens for use with the present invention can be produced using a variety of methods known to those of skill in the art. In particular, the antigens can be isolated directly from native sources, using standard purification techniques.

20 Alternatively, the antigens can be produced recombinantly using known techniques. See, e.g., Sambrook, Fritsch & Maniatis, *Molecular Cloning: A Laboratory Manual*, Vols. I, II and III, Second Edition (1989); *DNA Cloning*, Vols. I and II (D.N. Glover ed.

25 1985). Antigens for use herein may also be synthesized, based on described amino acid sequences, via chemical polymer syntheses such as solid phase peptide synthesis. Such methods are known to those of skill in the art. See, e.g., J. M. Stewart and J. D. Young, *Solid Phase Peptide Synthesis*, 2nd Ed., Pierce Chemical Co., Rockford, IL (1984) and G. Barany and R. B. Merrifield, *The Peptides: Analysis, Synthesis, Biology*, editors E. Gross and J. Meienhofer, Vol. 2, Academic Press, New York, (1980), pp. 3-254, for solid 30 phase peptide synthesis techniques; and M. Bodansky, *Principles of Peptide Synthesis*, Springer-Verlag, 35

Berlin (1984) and E. Gross and J. Meienhofer, Eds., *The Peptides: Analysis, Synthesis, Biology, supra*, Vol. 1, for classical solution synthesis.

Once obtained, the antigen of interest is
5 formed into a particulate vaccine composition as
described herein and administered to a subject,
generally along with an immunological adjuvant which
serves to enhance the immune response to the antigen.
As explained above, the adjuvant can be present in the
10 same or a separate composition and can be delivered
simultaneously with the vaccine composition, or prior
or subsequent to antigen delivery. Additionally,
adjuvant delivery can be at the same or a different
site.

15 Unfortunately, most known adjuvants are
highly toxic. Thus, the only adjuvant currently
approved for human usage is alum, an aluminum salt
composition.

20 A number of adjuvants are, however, used in animal
studies and several adjuvants for human use are
undergoing preclinical and clinical studies.

Surprisingly, adjuvants which are
generally considered too toxic for human use may be
administered with the present methods. Without being
25 bound by a particular theory, it appears that delivery
of adjuvants to the skin, using transdermal delivery
methods, allows interaction with Langerhans cells in
the epidermal layer and dendritic cells in the
cutaneous layer of the skin. These cells are
30 important in initiation and maintenance of an immune
response. Thus, an enhanced adjuvant effect can be
obtained by targeting delivery to or near such cells.
Additionally, because the top layers of the skin are
poorly vascularized, the amount of adjuvant entering
35 the systemic circulation is reduced, thereby reducing
the toxic effect. Furthermore, because skin cells are

constantly being sloughed, residual adjuvant is eliminated rather than absorbed. Moreover, less adjuvant can be administered than that delivered using conventional techniques such as intramuscular injection. Accordingly, the present invention may effectively be used with a large variety of adjuvants without concomitant toxicity. Such adjuvants include, without limitation, adjuvants formed from aluminum salts (alum), such as aluminum hydroxide, aluminum phosphate, aluminum sulfate, etc; oil-in-water and water-in-oil emulsion formulations, such as Complete Freunds Adjuvant (CFA) and Incomplete Freunds Adjuvant (IFA); adjuvants formed from bacterial cell wall components such as adjuvants including monophosphoryl lipid A (MPL) (Imoto et al. (1985) *Tet. Lett.* 26:1545-1548), trehalose dimycolate (TDM), and cell wall skeleton (CWS); adjuvants derived from ADP-ribosylating bacterial toxins, a group of potent toxins to humans, include diphtheria toxin, pertussis toxin (PT), cholera toxin (CT), the *E. coli* heat-labile toxins (LT1 and LT2), *Pseudomonas* endotoxin A, *C. botulinum* C2 and C3 toxins, as well as toxins from *C. perfringens*, *C. spiriforma* and *C. difficile*, particularly ADP-ribosylating bacterial toxin mutants such as CRM₁₉₇, a non-toxic diphtheria toxin mutant (see, e.g., Bixler et al. (1989) *Adv. Exp. Med. Biol.* 251:175; and Constantino et al. (1992) *Vaccine*); saponin adjuvants such as Quil A (U.S. Pat. No. 5,057,540), or particles generated from saponins such as ISCOMs (immunostimulating complexes); cytokines, such as interleukins (e.g., IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, etc.), interferons (e.g., gamma interferon), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), etc; muramyl peptides such as N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-

isoglutamine (nor-MDP) , N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2- (1'-2'-dipalmitoyl-sn-glycero-3 hydroxyphosphoryloxy)-ethylamine (MTP-PE) , etc.; adjuvants derived from the CpG family of 5 molecules, CpG dinucleotides and synthetic oligonucleotides which comprise CpG motifs (see, e.g., Krieg et al. *Nature* (1995) 374:546 and Davis et al. *J. Immunol.* (1998) 160:870-876) such as TCCATGACGTTCCCTGATGCT (SEQ ID NO:1) and 10 ATCGACTCTCGAGCGTTCTC (SEQ ID NO:2); and synthetic adjuvants such as PCPP (Poly[di(carboxylatophenoxy)phosphazene] (Payne et al. *Vaccines* (1998) 16:92-98). Such adjuvants are commercially available from a number of distributors 15 such as Accurate Chemicals; Ribi Immunochemicals, Hamilton, MT; GIBCO; Sigma, St. Louis, MO.

Once obtained, the adjuvant, with or without the antigen of interest, is formed into a particle suitable for transdermal delivery using any suitable 20 particle formation technique, such as air-drying (crystallization) freeze-drying (lyophilization), spray-coating or supercritical fluid techniques. The compositions may also be prepared as crystalline compositions, as described above.

Following their formation, the subject 25 particles are delivered transdermally to mammalian tissue using a suitable transdermal delivery technique. Various particle acceleration devices suitable for transdermal delivery of the substance of 30 interest are known in the art, and will find use in the practice of the invention. A particularly preferred transdermal delivery system employs a needleless syringe to fire solid drug-containing particles in controlled doses into and through intact skin and tissue. See, e.g., U.S. Patent No. 5,630,796 35 to Bellhouse et al. which describes a needleless

syringe (also known as "the PowderJect® needleless syringe device"). Other needleless syringe configurations are known in the art and are described herein.

5 The particles are administered to the subject in a manner compatible with the dosage formulation, and in an amount that will be effective to achieve the desired physiological response. Generally, the response generated will be 10 prophylactically and/or therapeutically effective. Thus, for example, if an antigen or vaccine composition is being delivered, the amount administered will be sufficient to generate an immune response. If an adjuvant is coadministered with the 15 antigen, it will be delivered in an amount sufficient to enhance the immune response to the coadministered antigen. It is readily apparent that the amount of the composition to be delivered depends on the particular substance administered, the subject to be 20 treated and the disease to be prevented or treated.

Generally about .5 μ g to 1000 μ g of adjuvant, more generally 1 μ g to about 500 μ g of adjuvant and most preferably about 5 μ g to about 300 μ g of adjuvant will be effective to enhance an immune 25 response of a given antigen. Thus, for example, for CpG, doses in the range of about .5 to 50 μ g, more preferably 1 to about 25 μ g, preferably 5 to about 20 μ g, will find use with the present methods. Similarly, for alum or PCPP, a dose of about 25 μ g to 30 about 500 μ g, preferably about 50 to about 250 μ g, and most preferably about 75 to about 150 μ g, will find use herein. For MPL, a dose in the range of about 10 to 250 μ g, preferably about 20 to 150 μ g, and most preferably about 40 to about 75 μ g, will find use with 35 the present methods.

Doses for other adjuvants can readily be determined by one of skill in the art using routine methods. The amount to administer will depend on a number of factors including the coadministered 5 antigen, as well as the ability of the adjuvant to act as an immune stimulator.

Similarly, if an antigen is administered transdermally, either in the same or a different 10 composition, generally 50 ng to 1 mg and more preferably 1 μ g to about 50 μ g of antigen, will be useful in generating an immune response. The exact 15 amount necessary will vary depending on the age and general condition of the subject to be treated, the severity of the condition being treated and the particular antigen or antigens selected, the site of 20 administration, as well as other factors. An appropriate effective amount can be readily determined by one of skill in the art upon reading the instant specification and through routine trials.

20 Dosage treatment may be a single dose schedule or a multiple dose schedule. For vaccine compositions, a multiple dose schedule is one in which a primary course of vaccination may be with 1-10 25 separate doses, followed by other doses given at subsequent time intervals, chosen to maintain and/or reinforce the immune response, for example at 1-4 months for a second dose, and if needed, a subsequent dose(s) after several months. The dosage regimen will also, at least in part, be determined by the need of 30 the subject and be dependent on the judgment of the practitioner. Furthermore, if prevention of disease is desired, the compositions are generally administered prior to primary infection with the pathogen of interest. If treatment is desired, e.g., 35 the reduction of symptoms or recurrences, the

compositions are generally administered subsequent to primary infection.

5 C. Experimental

Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present 10 invention in any way.

Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperatures, etc.), but some experimental error and deviation should, of course, be allowed for.

15

C.1 Particle Formulations and Particle Formation Techniques

Example 1

20 Particulate Vaccine Compositions

The following study was carried out to assess the effect that various excipients and particle formation processes have on the physical properties of 25 the resultant particulate vaccine compositions, and the immunogenicity of such reformulated vaccines. Diphtheria toxoid (dT), a purified subunit protein antigen, was selected for formulation with different excipients including mannitol (plus 30 polyvinylpyrrolidone (PVP)), sucrose, and trehalose. Two powder processing techniques, freeze-drying and air-drying (evaporative drying), were also compared. For each formulation tested, the resulting particulate 35 composition was classified in the following particle fractions: <20 μ m, 20-38 μ m, 38-53 μ m, and 53-75 μ m using 3" diameter stainless steel sieves.

Physical characterization of the particulate vaccine compositions included an assessment of size distribution, penetration energy, optical microscopy, scanning electronic microscopy, absolute density, pore size distribution, surface area analysis, and X-ray powder diffraction.

To determine immunogenicity of reformulated vaccine delivered with a needleless syringe device (e.g., a PowderJect® needleless syringe device) or by conventional syringe/needle injection, Balb/C mice (female, 6-8 weeks old) were vaccinated on weeks 0 and 4. Mice vaccinated with the PowderJect® needleless syringe device received 5 μ g of vaccine formulated with 1 mg of excipient. Control mice were injected 15 intraperitoneally with a conventional needle and syringe. Two weeks post boost, sera was collected and pooled from 8 mice, and antibodies to dT were determined by ELISA.

The result of the physical characterization 20 of the particulate compositions was as follows: (1) the air-drying process yielded high density crystal particles, while freeze-drying yielded relatively low-density amorphous powders; and (2) particle size distribution appeared to be independent of excipients 25 and the particle formation process used. The particles were fractionated by sieving into 5 sizes (<20 μ m, 20-38 μ m, 38-53 μ m, 53-75 μ m, and >75 μ m, and the mass distribution for each fraction is about equivalent.

30 The results of the immunogenicity assessment are depicted in Tables 1 and 2 below, and in Figure 1. As can be seen, immunogenicity between the various particulate vaccine compositions was very similar (among all formulations and different size fractions 35 for the same formulation). All of the particulate compositions delivered by needleless syringe elicited

higher antibody titers than the control group (conventional needle and syringe delivery). The trehalose excipient appeared to perform slightly better than the other formulations, and the 20-53 μ m fractions appeared to be more immunogenic (see Figure 1). However, both the $\leq 20\mu$ m and the $> 75\mu$ m particle fractions were more immunogenic than the control (aqueous compositions, conventional needle and syringe delivery). It can also be seen that needleless 10 syringe (PowderJect[®]) delivery of the crystalline vaccine composition particles resulted in a higher seroconversion rate than the control (conventional needle and syringe) injection method (see Table 2). In this regard, 97% (157 out of 162) of animals 15 developed serum antibodies after a single PowderJect[®] vaccination, while the seroconversion rate was 37.5% (3 out of 8) for the control group.

20

25

30

35

Table 1

IgG Antibodies to dT (Pooled Serum)

	Excipient	Particle Formation Method	Particle Size (μm)	Delivery Method	Prime	Boost
5	saline	control	-	syringe and needle	1220	111600
10	trehalose	Air-dry	<20	PJ*	1550	616380
	trehalose	Air-dry	20-38	PJ	5460	1390700
	trehalose	Air-dry	38-53	PJ	7295	1282100
	trehalose	Air-dry	53-75	PJ	2690	577080
	trehalose	Air-dry	>75	PJ	2310	620460
15	trehalose/mannitol	Freeze-dry	20-38	PJ	1590	441140
	trehalose + mannitol	Freeze-dry	38-53	PJ	4590	686540
	trehalose + mannitol	Freeze-dry	53-75	PJ	2850	657780
	trehalose + mannitol	Freeze-dry	>75	PJ	1940	282630
20	sucrose	Air-dry	20-38	PJ	4650	1160220
	sucrose	Air-dry	38-53	PJ	3040	515760
	sucrose	Air-dry	53-75	PJ	1750	372160
	sucrose	Air-dry	>75	PJ	5230	759240
25	sucrose	Freeze-dry	20-38	PJ	1525	618100
	sucrose	Freeze-dry	38-53	PJ	2950	614780
	sucrose	Freeze-dry	53-75	PJ	7830	802850
	sucrose	Freeze-dry	>75	PJ	1800	780710
30	Mannitol/PVP	Freeze-dry	20-38	PJ	3700	494330
	Mannitol/PVP	Freeze-dry	38-53	PJ	2810	609940
	Mannitol/PVP	Freeze-dry	53-75	PJ	4580	935700
35	Mannitol/PVP	Freeze-dry	>75	PJ	1590	447630

PJ = PowderJect®

Note: Week 0 sera had a titer <200. The dT dose for all vaccination is 5 μg .

Table 2

Excipient	Particle Formulatio n Method	Seroconverted/Total Animals				
		<20	20-38	38-53	53-75	>75
Trehalose	Air-Dry	4/4	7/8	7/8	7/8	8/8
Trehalose + Mannitol	Freeze-Dry	-	8/8	8/8	8/8	7/8
Sucrose	Air-Dry	-	7/8	8/8	7/7	7/7
Sucrose	Freeze-Dry	-	8/8	8/8	8/8	6/8
Mannitol/P VP	Freeze-Dry	-	8/8	7/8	8/8	7/8
DT/injecti on	-	3/8				

15 C.2 Formation and Assessment of Crystalline
Vaccine Compositions

Example 2

Formulation of Crystalline Vaccine Compositions

20 A number of conventional vaccine
compositions were crystallized using the following
methods.

25 *Pneumococcus capsular polysaccharide #14*
(CP14) was obtained as a lyophilized powder from the
ATCC. A volume of 1 ml of Water for Injection was
dispensed into a 2 mg vial of CP14, and the resulting
suspension was continuously mixed at 4°C overnight as
specified by the manufacturer's instructions. 100 μ l
30 aliquots of the mixture were made and frozen until
needed. A quantity of 99.5 mg trehalose (Sigma)
powder was weighed and mixed with defrosted aliquots
of the CP14 suspension to provide a 500 μ g total of
CP14. Approximately 1200 μ l of Water for Injection
was used to dissolve the CP14/trehalose mixture, and
the solution was thoroughly mixed. 100 μ l aliquots of
35 the solution were then dispensed onto the surface of
weigh boats, and placed in the constant airflow

provided by a fume hood. The droplets were then dried evaporatively over the next 1-2 days to form a crystalline product. The crystals were removed from the weigh boats and ground lightly using mortar and pestle.

5 One standard adult vial of Engerix-B® (Smith Kline Beecham), which contains 20 µg of the HepB surface antigen adsorbed onto 0.5 mg aluminum hydroxide (alum) was combined with 10 mg trehalose, 10 and the resulting solution mixed thoroughly. 100 µl droplets were dispensed onto weigh boats, and desiccated as described herein above. After approximately 36 hours of evaporative drying, crystalline vaccine residues were carefully removed 15 from the weigh boats using a spatula. The crystalline composition was then ground lightly using mortar and pestle until the larger crystals had been visibly reduced in size. 1.25 ± 0.25 mg aliquots were dispensed into drug cassettes, providing a nominal 20 dose of 2.5 µg of the Hepatitis B surface antigen.

A quantity of Hepatitis B surface antigen (HbsAg), purified from human plasma, was obtained (Biodesign International). The HbsAg was combined with trehalose and dI water solution. The resulting solution was gently mixed, poured into a glass petri dish and allowed to air-dry for 2 days under a fume hood. Further drying was carried out for an additional day in a desiccator (Nalgene Plastic desiccator) which was purged with N₂ gas. The dried solid composition was collected by scraping and then comminuted using a mortar and pestle. The resultant dry powder was weighed, and the amount of dry material for each dose was determined by dividing the total weight by the number of doses formulated. Particle size distribution of the formulated HbsAg vaccine composition varied over a broad range (1-100 µm).

Typically, each dose required about 1-2 mg of dry mass, with a weighing variation of about 10% or less.

A quantity HibTITER® (Wyeth Lederle), which is comprised of a PRP-CRM197 conjugate vaccine composition, was obtained. The composition contains polyribosyl ribose phosphate (polysaccharide from type b *Haemophilus influenzae*) conjugated to the CRM197 mutant diphtheria toxin carrier, and is referred to herein as the "Hib conjugate vaccine composition." 5
10 The Hib conjugate vaccine composition was combined with trehalose, and the resulting solution mixed thoroughly. The solution was then dispensed onto weigh boats, and desiccated as previously described. After evaporative drying, crystalline vaccine residues 15 were obtained from the weigh boats, and the crystalline composition was ground lightly using mortar and pestle, and appropriate dosages thereof were measured into cassettes for delivery from a needleless syringe.

20 A quantity of influenza virus, PR8 strain, was obtained from Spafas (Storrs, CT). In addition, a quantity of influenza virus, Aichi strain, was obtained from Dr. Yoshihero Kawaoka, Veterinary School, University of Wisconsin (Madison, Wisconsin). 25
Each virus was inactivated by standard formalin treatment (1:4,000, 48 hours at 4°C). Inactivated virus (either PR8 or Aichi) was then combined with trehalose and dI water solution. The resulting solution was gently mixed, poured into a glass petri dish and allowed to air-dry for 2 days under a fume hood. Further drying was carried out for an additional day in a desiccator (Nalgene Plastic desiccator) which was purged with N₂ gas. The dried solid composition was collected by scraping and then 30
35 comminuted using a mortar and pestle. The resultant dry powder was weighed, and the amount of dry material

for each dose was determined by dividing the total weight by the number of doses formulated. Particle size distribution of the formulated whole-inactivated virus vaccine composition varied over a broad range (1-100 μm). Typically, each dose required about 1-2 mg of dry mass, with a weighing variation of about 10% or less.

A quantity of Diphtheria toxoid was obtained (Accurate Chemical & Scientific Corp., manufactured by Statems Serum Institute, Denmark). The toxoid was combined with trehalose and dI water solution. The resulting solution was gently mixed, poured into a glass petri dish and allowed to air-dry for 2 days under a fume hood. Further drying was carried out for an additional day in a desiccator (Nalgene Plastic desiccator) which was purged with N_2 gas. The dried solid composition was collected by scraping and then comminuted using a mortar and pestle. The resultant dry powder was weighed, and the amount of dry material for each dose was determined by dividing the total weight by the number of doses formulated. Particle size distribution of the formulated Diphtheria toxoid vaccine composition varied over a broad range (1-100 μm). Typically, each dose required about 1-2 mg of dry mass, with a weighing variation of about 10% or less.

Example 3

Vaccination with the Crystalline Vaccine Compositions

Devices: Unless otherwise noted, the needleless syringe delivery devices used in skin delivery studies (either the PowderJect® ND device series or Oral PowderJect® device series) were obtained from PowderJect Technologies, Ltd., Oxford, UK. The PowderJect® ND device is generally described

in commonly owned U.S. Patent No. 5,630,796. The Oral PowderJect® device is generally described in commonly owned International Publication No. WO 96/20022. The gas cylinders in the devices used herein were 5 typically filled with helium gas between 40 and 60 bar pressure, although anywhere from 30 to 80 bar pressure can also be used. In operation, compressed helium in the gas cylinder is released upon actuation of the device, rupturing the membranes of the particle-containing payload cassette. A supersonic condition 10 is created, and the resulting high velocity gas flow propels the particles as projectiles into the target tissue surface. Varying the pressure of the helium gas in the gas cylinder can control the depth of 15 penetration. For conventional needle and syringe delivery, disposable syringes were fitted with 26.5 gauge needles.

Mice and vaccination: Female Balb/c mice or Swiss Webster mice, 7 weeks of age were purchased from 20 an authorized vendor (e.g., HSD) and acclimated for 1 week at a mouse facility before vaccination. Mice were anesthetized by an intraperitoneal (IP) injection of 100mg/kg ketamine mixed with 10 mg/kg xylazine, and the abdominal skin at the target site was depilated by 25 shaving. The needleless syringe device was gently pressed against the vaccination site and abutted. A typical immunization regime consisted of two vaccinations, four weeks apart, with blood collection via retro-orbital bleeding under anesthesia prior to 30 each vaccination and two weeks post boost.

ELISA: In general, antibody response to the reformulated vaccines was determined by an ELISA. A 35 96-well plate was coated with 0.1 μ g of detecting antigen in PBS per well overnight at 4°C. The plates were washed 3 times with TBS containing 0.1% Brij-35, and incubated with test sera diluted in PBS containing

1% BSA for 1.5 hours. A serum standard, which contains high level of antibodies to specific antigen, was added to each plate and used to standardize the titer in the final data analysis. The plates were then 5 washed and incubated with biotin-labeled goat antibodies specific for mouse immunoglobulin IgG or IgG subclasses (1:8,000 in PBS, Southern Biotechnology) for 1 hr at room temperature. Following three additional washes, the plates were 10 incubated with strepavidin-horseradish peroxidase conjugates (1:8,000 in PBS, Southern Biotechnology) for 1 hr at room temperature. Finally, the plates were washed and developed with a TMB substrate kit (obtained from Bio-Rad, Richmond, CA). Endpoint 15 titers of the test sera were determined using the Softmax Pro 4.1 program (Molecular Devices) as the calibrated highest dilution with an A_{450} that exceeded the mean background by 0.1. Mean background absorbence was determined by wells that received all 20 reagents but test sera.

1. Vaccination with crystalline pneumococcus capsular polysaccharide #14 (CP14). Experimental groups of Balb/c mice were randomly 25 formed based on the route of administration (intraperitoneal (IP) or intradermal (ID)), and formulation, as shown in the test matrix of Table 3. The mice were given a prime and a boost four weeks after the prime using the above-described crystalline 30 CP14 vaccine composition, and then bled 10 days after the boost. For the boost, the mice were anesthetized with an injection of a mouse anesthetic to enable retro-orbital bleeding prior to booster 35 administration. For needless syringe delivery, fur was removed by clipping, and delivery was carried out using 60 bar gaseous pressure from the PowderJect®.

needleless syringe delivery device. Serum samples are then analyzed to determine antibody titers to pneumococcus.

5

Table 3

Group #	n	Delivery Route	Pressure (bar)	Formulation	CP dose (μ g)
I	3	N/S-IP	---	CP14 + Trehalose with alum	5
II	3	N/S-IP	---	CP14 + Trehalose	5
III	3	N/S-IP	---	CP14 + Alum	5
IV	4	PJ-ID	60	CP14 + Trehalose	5
V	4	PJ-ID	60	CP14 + Trehalose	10
VI	4	PJ-ID	60	CP14	50
VII	2	PJ-ID	60	Trehalose only	0

2. Vaccination with the crystalline Hepatitis B (HepB) surface antigen vaccine composition. The crystalline HepB vaccine composition (as described above) was administered (in 2.5 μ g carbohydrate doses) to mice as follows. Eight Balb/c mice were divided into two cohorts based on the vaccine composition and delivery technique used: (1) intraperitoneal using a needle-syringe to deliver conventional (liquid) Engerix-B® vaccine composition (n=2); and (2) intradermal using a PowderJect® needleless syringe to deliver the crystalline vaccine composition (n=6). Delivery pressure from the PowderJect® device ranged from 40 to 60 bar. All animals were boosted three weeks after priming, and bled 10 days following the boost.

Antibody titers to the Hepatitis B surface antigen were then determined by ELISA using the blood samples obtained 10 days post administration of the

boost. The results are depicted below in Table 4. Titters greater than about 10 mIU/mL are considered sero-protective. As can be seen, 5 out of 6 of the animals receiving the crystalline composition via needleless syringe were protected by the crystalline vaccine composition.

Table 4

Mouse #	Delivery Route	Po (bar)	Comments
1	Needle-syringe (IP)	---	---
2	Needle-syringe (IP)	---	---
3	PowderJect® (ID)	40	Prime: poor delivery
4	PowderJect® (ID)	40	Prime: good
5	PowderJect® (ID)	40	Prime: OK
6	PowderJect® (ID)	60	Prime: OK
7	PowderJect® (ID)	60	Prime: OK
8	PowderJect® (ID)	60	Prime: microbleeds

A subsequent boost using the same crystalline HepB surface antigen vaccine composition was sufficient to significantly raise all antibody titers in the animals receiving the needleless injection (via the PowderJect® device), such that all 6 animals were protected by the crystalline vaccine composition.

3. Vaccination with the crystalline *Haemophilus influenzae* polyribosyl ribose phosphate conjugate vaccine composition (Hib conjugate). The above-described crystalline Hib conjugate vaccine composition was administered to mice as follows. Swiss Webster mice were divided into experimental groups based on administration technique, dosage, and

formulation: (Group 1, 2 μ g (PRP carbohydrate) of a liquid Hib conjugate vaccine composition delivered IP using conventional needle and syringe, n=3); (Group 2, 2.5 μ g (PRP carbohydrate) of the crystalline Hib conjugate vaccine composition delivered to skin using the PowderJect[®] needleless syringe, n=6); and (Group 3, control (naive), n=3). All vaccinated animals received a prime, followed by a boost at 4 weeks after prime. Serum samples were collected 2 weeks after boost, pooled and antibody titers to immobilized PRP-CRM197 were determined using ELISA.

Due to observed differences in binding of human and mouse anti-Haemophilus polysaccharide (HbPs) antibodies, the HbO-HA ELISA described by Phipps et al. (1990) *J. Immunol. Methods* 135:121-128, was adapted for use in testing mouse sera. The assay conditions adapted for the measurement of mouse anti-HbPs antibodies are noted in Table 5 below. All other assay conditions are as described by Phipps et al.

20

Table 5

	Human	Mouse
Antigen Coating Buffer	PBS	50 mM HEPES
Serum Dilution Buffer	PBS/0.3% Tween 20/0.01M EDTA	50 mM HEPES/0.1% Brij 35/1% FBS
Serum Incubation Time & Temp	60 minutes at room temperature	Overnight @ 4°C
Secondary Antibody Conjugate	anti-human Ig' AP	anti-mouse IgG' AP
Secondary Conjugate Buffer	PBS/0.05% Tween 20	50 mM HEPES/0.1% Brij 35/1% FBS
Wash Buffer	PBS/0.1% Tween 20	50 mM HEPES/0.1% Brij 35

35 *Evaluation has shown that quantitative antibody values in mouse sera measured using this ELISA are higher than those measured using a radio-antigen binding assay (RABA) especially for sera with titers less than 10 μ g/mL in the RABA.

5 The results of the ELISA are depicted below in Table 6. As can be seen, the crystalline vaccine composition gave comparable results with the conventional vaccine composition.

Table 6 Immunogenicity of Hib Conjugate in Mice					
	Immunogen	Number of Mice	$\mu\text{g X}$ Doses	Mode of Delivery	ELISA Titer to PRP-CRM197 IgG IgM
10	PRP-CRM197	3	2 $\mu\text{g X 2}$	Needle, IP	24,300 <100
15	PRP-CRM197	6	2.5 $\mu\text{g X 2}$	Dry Powder, Skin	24,300 <100
	None	3	-	Naive	<100 <100

20 In order to further characterize the immune response in the animals receiving the Hib conjugate vaccine composition, the following study was carried out. Three experimental groups of 6 mice each were assembled as follows: (Group 1, 2 μg dose (PRP carbohydrate) of the crystalline Hib conjugate vaccine composition delivered intradermally using the PowderJect® needleless syringe device); (Group 2, 2 μg dose (PRP carbohydrate) of a conventional liquid Hib conjugate vaccine composition delivered 25 intraperitoneally (IP) using needle and syringe); and (Group 3, control). The animals were primed, and then boosted four weeks later. Sera collected 2 weeks after boost were pooled, and serial dilutions were 30 assayed using the above-described ELISA techniques. In a first ELISA, the PRP-CRM197 conjugate was used as the capture phase. The results of this first ELISA are reported below in Table 7, and depicted in Figure 2. As can be seen, the crystalline composition 35

provided a substantially identical response relative to the conventional vaccine delivery.

Table 7
Vaccine Response

Table 7
Vaccine Response

	Dilution	IP*	PJ*	Naive
1	100	1.09 (0.024)	1.131 (0.012)	0.058 (0.019)
2	300	0.934 (0.053)	0.989 (0.003)	0.02 (0.016)
3	900	0.8015 (0.0445)	0.774 (0.004)	0.02 (0.014)
4	2700	0.449 (0.071)	0.502 (0.002)	0.015 (0.013)
5	8100	0.279 (0.006)	0.277 (0.004)	0.01 (0.001)
6	24300	0.129 (0.0315)	0.115 (0.005)	0.009 (0.001)
7	72900	0.0545 (0.0018)	0.041 (0.001)	0.017 (0.008)
8	21870 0	0.109 (0.005)	0.062 (0.02)	0.073 (0.021)

* (IP) = intraperitoneal needle & syringe delivery, (PJ) = PowderJect® needleless syringe delivery.

In order to assess the specificity of the immune response, a second ELISA was performed using diphtheria toxoid as the capture phase. In this regard, CRM197 is a mutant form of the diphtheria toxoid, but CRM197 is highly cross-reactive. Binding of antisera to the diphtheria toxin was thus used to assess response toward the CRM197 carrier protein.

The results are reported below in Table 8, and are depicted in Figure 3. Again, the crystalline vaccine composition gave comparable results to the conventional vaccine composition.

Table 8

	1	2	3	4
	Dilution	Naive	PJ*	IP*
5	100	0.21	1.856	1.8
10	300	0.102	1.846	1.64
15	900	0.117	1.688	1.616
20	2700	0.084	1.38	1.194
25	8100	0.072	0.931	0.797
30	24300	0.077	0.578	0.512
35	82900	0.072	0.3	0.269
40	248700	0.072	0.165	0.17

* (IP) = intraperitoneal needle & syringe delivery, (PJ) = PowderJect® needleless syringe delivery.

15 In order to detect PRP-specific antibody response, a third ELISA was performed using a PRP-human serum albumin (PRP-HSA) conjugate as the solid (capture) phase. Since the mice had not been immunized with the PRP-HSA conjugate, and had not been 20 exposed to HSA in other contexts, antibody binding is due to the presence of anti-PRP antibodies. The results of this ELISA are reported in Table 9 below, and depicted in Figure 4. As can be seen, the crystalline and the conventional (liquid) Hib 25 conjugate vaccine compositions gave comparable results.

30

35

Table 9

	1	2	3	4
	Serum Dilution	Naive	PJ*	IP*
1	200	0.184	1.244	1.649
2	400	0.143	0.731	0.956
3	800	0.113	0.455	0.625
4	1600	0.097	0.259	0.322
5	3200	0.075	0.159	0.171
10	6400	0.064	0.105	0.094
	12800	0.064	0.078	0.068

* (IP) = intraperitoneal needle & syringe delivery, (PJ) = PowderJect® needleless syringe delivery.

In order to determine antibody responses to descending doses of Hib conjugate vaccine following PowderJect® delivery or conventional needle-syringe injection, the following study was carried out. Swiss Webster mice (female, 6-8 weeks old) were vaccinated twice (prime and boost) with either a liquid Hib conjugate vaccine composition or the crystalline Hib conjugate vaccine composition at 4 week-intervals. Four doses (1 μ g, 0.2 μ g, 0.04 μ g, and 0.01 μ g per dose) of the Hib conjugate composition were tested. For needleless syringe vaccination, each vaccine dose was formulated with 1 mg of trehalose. Control mice were injected intraperitoneally (IP) with a conventional needle and syringe. Blood samples were collected prior to each vaccination, and 2-weeks post boost. Antibodies to PRP and CRM197 were assayed by ELISA as described above. Sera collected 2 weeks post boost were pooled, and the pooled sera were assayed using the above-described ELISA techniques. The results are depicted in Figure 5. As can be seen, at the 1 μ g dose, the IgG titers appeared to be comparable between the mice immunized by PowderJect® needleless syringe

delivery and the mice immunized using conventional needle and syringe. In the 0.01-0.2 μ g dose range, antibody levels in the mice immunized using the PowderJect® device appeared to be higher than mice in the corresponding needle and syringe delivery groups. In addition, antibody responses to CRM197 were also measured in the immunized animals. Dose-dependent responses were seen in the groups immunized with the PowderJect® device (see Figure 5). Control mice (those animals which were immunized by conventional needle and syringe injection) responded to vaccinations at the higher (1 μ g and 0.2 μ g) doses, but not at the lower doses. These data indicate that transdermal immunization of particulate vaccine compositions using the PowderJect® needleless syringe device is more effective than delivery of liquid vaccine compositions using conventional needle and syringe injection techniques, especially for delivering antigens at low doses.

In order to assess the duration of immunity provided by the crystalline Hib conjugate vaccine composition, the following study was carried out. Swiss Webster mice (female, 6-8 weeks old) were vaccinated with two doses (prime and boost) of the Hib conjugate crystalline vaccine composition at 4 week-intervals. For PowderJect® needleless syringe vaccinations, 1 μ g of vaccine was formulated with 1mg of the trehalose excipient. As controls, mice were injected IP with 5 μ g of a liquid Hib conjugate vaccine composition using conventional needle and syringe delivery techniques. Blood samples were collected before each vaccination, 2 weeks post boost, and monthly thereafter. Antibodies specific to PRP and CRM197 were assayed using the above-described ELISA techniques. The results of the ELISA assays are depicted in Figures 6A and 6B. As can be seen,

PowderJect® delivery of 1 μ g of the crystalline Hib conjugate vaccine composition generated levels of serum antibodies to PRP and CRM197 equivalent to that elicited by 5 μ g of conjugate administered by conventional needle and syringe injection. Antibodies peaked two weeks after Boost and lasted for 8 months without significant reduction. The results indicate that transdermal delivery of the particulate vaccine composition with the PowderJect® device elicits a long-lasting serum antibody response that is comparable to conventional needle and syringe delivery of a liquid vaccine composition.

4. Vaccination with the crystalline inactivated influenza virus vaccine composition.

In order to determine antibody responses to descending doses of influenza vaccine following PowderJect® delivery of a crystalline vaccine composition or conventional needle and syringe injection of a liquid vaccine composition, the following study was carried out. Five experimental groups of Balb/c mice (female, 6-8 weeks old) were established in order to assess five different doses of the above-described crystalline inactivated influenza virus (Aichi strain) composition. The five groups received vaccinations at weeks 0, 4 and 10.5 with 25 μ g, 5 μ g, 1 μ g, 0.2 μ g, or 0.04 μ g, respectively, of the inactivated influenza virus. For PowderJect® transdermal administration, each dose of the vaccine was formulated with 1 mg of trehalose. Five groups of control mice were also established. These control mice received vaccinations at weeks 0, 4 and 10.5 with 25 μ g, 5 μ g 1 μ g, 0.2 μ g, or 0.04 μ g, respectively, of inactivated influenza virus in a liquid vaccine composition (administered IP by conventional needle and syringe injection). Two weeks after the third vaccination, sera from 8 mice were

collected and pooled, and an ELISA determined antibodies to influenza virus.

Two weeks post boost, antibody titers in pooled sera were determined against the Aichi virus 5 using the above-described ELISA techniques. The results of the ELISA are depicted in Figure 7. As can be seen, there was a dose dependent antibody response for both the PowderJect® transdermal delivery of the crystalline composition and the conventional needle 10 and syringe injection of the liquid composition. However, at the same doses of vaccine, the PowderJect® vaccination elicited higher antibody titers than the needle and syringe injection, indicating that 15 PowderJect® delivery of a crystalline vaccine to the skin improves vaccine performance.

Pooled sera were also tested for hemagglutination inhibition activity (HI). The results of the HI activity assay are depicted below in Table 10. As can be seen, there were dose-dependent 20 HI titers in the sera from the vaccinated animals. HI titers from animals receiving PowderJect® delivery and conventional needle and syringe injection are similar at the higher vaccine doses (25 μ g, 5 μ g and 1 μ g). However, at the lower vaccine doses (0.2 μ g and 0.04 μ g) 25 HI titers were only elicited in animals receiving the crystalline composition from the PowderJect® system.

30

35

Table 10

HI Antibody Titer in Pooled Week 12 Sera

Influenza Vaccine (μg)	PowderJect® Delivery	Syringe and Needle Injection
25	40	80
5	20	20
1	20	10
0.2	10	-
0.04	10	-

In order to assess protection against a subsequent influenza virus challenge, the vaccinated animals were challenged on week 12 by intranasal instillation of 1×10^6 PFU (100X LD₅₀) of a mouse adapted Aichi influenza virus. More particularly, ten days after the final immunization, mice were anesthetized by an intraperitoneal injection of 100mg/kg ketamine mixed with 10 mg/kg xylazine. 1×10^6 particle forming units (PFUs) of influenza virus in 50 μl of saline was slowly instilled into the opening of nasal cavity. The mice naturally inhaled the liquid. Animals were then allowed to recover. Body weight of the animals was taken prior to challenge and daily post challenge for 14 days. Animals were monitored daily for symptoms and survival. Animals that lost 25% of pre-challenge body weight and became moribund were euthanized by CO₂.

Survival and body weight loss were recorded daily for 14 days after the challenge. All animals lost weight during days 3-7 after challenge. Survivors gained their weight back by day 14. The results of the protection study are depicted below in Table 11. The survival statistics for animals receiving the vaccine compositions containing 25μg and 5μg of the inactivated virus are depicted in Figures

8A and 8B, respectively. As can be seen, at the 25 μ g dose, PowderJect® administration of the crystalline composition gave better protection against mortality than the liquid composition when delivered via conventional needle and syringe injection. A correlation between antibody response and survival was seen in that the animals that died had lower antibody titers. At the 5 μ g dose, PowderJect® administration of the crystalline composition provided partial protection against mortality, compared with no protection in the animals receiving the same dose by conventional needle and syringe injection. Therefore, transdermal delivery of the crystalline composition via the PowderJect® device provided better protection than conventional needle and syringe injection.

Table 11

Influenza Challenge Experiment

Influenza Vaccine (μ g)	Survival/Total	
	PowderJect®	Syringe & Needle
25	7/8	5/8
5	3/8	0/8
1	0/8	0/8
0.2	0/8	0/8
0.04	0/8	0/8

Note: Mice received 3-vaccinations on weeks 0, 4 and 10, were challenged with 100X LD50 of a homologous mouse adapted influenza virus on week 12. Data represents protection for 2 weeks post-challenge.

5. Vaccination with the crystalline Diphtheria toxoid (dT) vaccine composition. In order to determine antibody responses to descending doses of dT vaccine following PowderJect® delivery of the crystalline composition, and to compare these antibody

responses with those attained from conventional needle and syringe injection, the following study was carried out. Two experimental groups of Balb/c mice (female, 6-8 weeks old) were established. The animals received 5 vaccinations at weeks 0 and 4 with a liquid dT vaccine composition (delivered by needle and syringe injection) or with a crystalline vaccine composition (delivered by the PowderJect® needleless syringe device). Two vaccine doses were tested, that is 10 vaccine compositions containing 5 μ g and 1 μ g of the dT toxoid. For PowderJect® vaccination, each dose of vaccine was formulated with 1 mg of trehalose. Control mice were injected IP with a conventional needle and syringe. Two weeks after the final 15 vaccinations, sera were collected and pooled from 8 mice, and antibodies to dT were determined by the above described ELISA techniques.

The results of the ELISA are depicted below 20 in Table 12. As can be seen, transdermal delivery of the crystalline composition containing 1 μ g of dT resulted in a serum antibody response that was 15-fold higher than in animals receiving the same dose of a liquid vaccine composition by conventional needle and syringe injection. This indicates that particle- 25 mediated skin delivery is an effective way to administer the dT vaccine. At the 5 μ g dose, serum antibody levels in animals receiving the crystalline composition by the PowderJect® device were similar to those seen in animals receiving the liquid composition 30 by conventional needle and syringe injection.

Table 12

IgG Titer to dT in Pooled Week 6 Sera Determined by ELISA		
dT (μg)	Serum IgG Titer (PowderJect®)	Serum IgG Titer (Syringe/Needle)
1	8130	570
5	188830	149560

C.3 Formation and Assessment of Particulate Adjuvant Compositions

10

Example 4

Formulation of Particulate Adjuvant Compositions

A number of conventional adjuvant compositions were formulated in particulate (powder) form pursuant to the methods of the invention. These and other adjuvants can be readily reformulated as powders using any number of conventional particle-forming processes. Suitable excipients for the adjuvant compositions include trehalose, sucrose, agarose, mannitol, or a mixture of these and/or other sugars. Particle formation techniques can include air-drying, freeze-drying, spray-coating, and supercritical fluid methods. However, all adjuvant formulations used in the following experiments were prepared using the crystallization methods of the present invention (particularly as described above in Sections C.1 and C.2) unless expressly noted otherwise.

The particulate adjuvant compositions were produced as follows.

A quantity of aluminum hydroxide and aluminum phosphate adjuvant ("Alum adjuvant") was obtained (manufactured by Superfow Biosector a/s, obtained from Accurate Chemical and Scientific Corp.). The Alum adjuvant was combined with trehalose and dI water solution. The resulting solution was gently

mixed, poured into a glass petri dish and allowed to air-dry for 2 days under a fume hood. Further drying was carried out for an additional day in a desiccator (Nalgene Plastic desiccator) which was purged with N₂ gas. The dried solid composition was collected by scraping and then comminuted using a mortar and pestle. The resultant dry powder was weighed, and the amount of dry material for each dose was determined by dividing the total weight by the number of doses formulated. Particle size distribution of the formulated Alum adjuvant composition varied over a broad range (1-100 μ m). Typically, each dose required about 1-2 mg of dry mass, with a weighing variation of about 10% or less.

A quantity of the MPL adjuvant (monophosphoryl Lipid A purified from *S. minnesota* R595) was obtained (RIBI ImmunoChem Research, Inc.). The MPL adjuvant was combined with trehalose and DI water solution. The resulting solution was gently mixed, poured into a glass petri dish and allowed to air-dry for 2 days under a fume hood. Further drying was carried out for an additional day in a desiccator (Nalgene Plastic desiccator) which was purged with N₂ gas. The dried solid composition was collected by scraping and then comminuted using a mortar and pestle. The resultant dry powder was weighed, and the amount of dry material for each dose was determined by dividing the total weight by the number of doses formulated. Particle size distribution of the formulated MPL adjuvant composition varied over a broad range (1-100 μ m). Typically, each dose required about 1-2 mg of dry mass, with a weighing variation of about 10% or less.

A quantity of CpG adjuvant (20mer synthetic oligonucleotides, CpG-1: ATCGACTCTCGAGCGTTCTC, SEQ ID NO. 1 and CpG-2: TCCATGACGTTCTGATGCT, SEQ ID NO. 2)

was obtained (GIBCO-BRL). The CpG adjuvant was combined with trehalose and dI water solution. The resulting solution was gently mixed, poured into a glass petri dish and allowed to air-dry for 2 days 5 under a fume hood. Further drying was carried out for an additional day in a desiccator (Nalgene Plastic desiccator) which was purged with N₂ gas. The dried solid composition was collected by scraping and then comminuted using a mortar and pestle. The resultant 10 dry powder was weighed, and the amount of dry material for each dose was determined by dividing the total weight by the number of doses formulated. Particle size distribution of the formulated CpG adjuvant composition varied over a broad range (1-100 μm). 15 Typically, each dose required about 1-2 mg of dry mass, with a weighing variation of about 10% or less.

A quantity of PCPP adjuvant (a synthetic polymer- poly[di(carboxylatophenoxy)phosphazene]) was obtained (Virus Research Institute). The PCPP 20 adjuvant was combined with trehalose and dI water solution. The resulting solution was gently mixed, poured into a glass petri dish and allowed to air-dry for 2 days under a fume hood. Further drying was carried out for an additional day in a desiccator 25 (Nalgene Plastic desiccator) which was purged with N₂ gas. The dried solid composition was collected by scraping and then comminuted using a mortar and pestle. The resultant dry powder was weighed, and the amount of dry material for each dose was determined by 30 dividing the total weight by the number of doses formulated. Particle size distribution of the formulated PCPP adjuvant composition varied over a broad range (1-100 μm). Typically, each dose required about 1-2 mg of dry mass, with a weighing variation of 35 about 10% or less.

Example 5Vaccination with the Particulate Adjuvant Compositions

The devices used to deliver the particulate compositions (i.e., the PowderJect® needleless syringe devices) and the control (liquid) compositions (i.e., conventional needle and syringe) are as described above in Example 3. The experimental animals (female Balb/c mice) were handled as above, vaccine compositions were also delivered as described above, and the same ELISA techniques as described above were used in the following studies. The crystalline and control (liquid) vaccine compositions which were used in the following studies were the inactivated whole influenza virus (Aichi strain) compositions and the Diphtheria toxoid compositions described above in Example 2. Viral challenge studies (in the influenza studies) with the mouse-adapted Aichi influenza strain were carried out as described above in Example 3.

20

1. Vaccination studies with the particulate Alum adjuvant composition. The particulate Alum adjuvant composition was used as an adjuvant with the crystalline inactivated influenza vaccine composition and delivered via the PowderJect® needleless syringe delivery system. Mice were vaccinated with 5 μ g or 1 μ g of the crystalline inactivated influenza vaccine composition either with or without 100 μ g of the particulate Alum adjuvant composition. Control animals were vaccinated subcutaneously ("S/C") with aqueous formulations of the same vaccine/ adjuvant compositions using a conventional needle and syringe. After three vaccinations were carried out (at weeks 0, 4, and 10.5), week 12 sera were pooled from 8 mice, and

antibodies to influenza virus were determined by the above-described ELISA techniques.

The results of the ELISA study are reported in Table 13 below. Following skin delivery of Alum-adjuvanted influenza vaccine, the serum antibody response was significantly higher when compared with animals receiving the same dose of vaccine without the adjuvant. Serum antibody levels in the animals that received the particulate compositions via the PowderJect® device were similar to those from animals that were vaccinated with the same vaccine/adjuvant composition using conventional techniques.

Accordingly, the Alum adjuvant can be delivered to the skin in powder form to enhance the immunogenicity of an influenza vaccine.

Table 13

Total IgG Titer in Pooled Week 12 Sera Determined by ELISA

Aluminum (μ g)	Influenza Vaccine (μ g)	Serum IgG Titer (PowderJect®)	Serum IgG Titer (Syringe/Needle)
none	5	6790	1505
none	1	873	804
100	5	27180	19198
100	1	2539	9966

In order to assess protection against a subsequent influenza virus challenge, the vaccinated animals were challenged on week 12 by intranasal instillation of 1×10^6 PFU (100X LD₅₀) of a mouse adapted Aichi influenza virus. More particularly, ten days after the final immunization, mice were anesthetized by an intraperitoneal injection of 100mg/kg ketamine mixed with 10 mg/kg xylazine. 1×10^6 particle forming units (PFUs) of influenza virus in 50 μ l of saline was slowly instilled into the opening of nasal cavity. The mice naturally inhaled the liquid.

Animals were then allowed to recover. Body weight of the animals was taken prior to challenge and daily post challenge. Animals were monitored daily for symptoms and survival. Animals that lost 25% of pre-
5 challenge body weight and became moribund were euthanized by CO₂.

Survival and body weight loss were recorded daily for 14 days after the challenge. These results are reported below in Table 14 and depicted in Figure 9. As can be seen, the survival rate in mice receiving the Alum-adjuvanted vaccine was much greater than that in mice receiving the vaccine alone. PowderJect® delivery of the particulate Alum-adjuvanted influenza vaccine composition offered better protection against weight loss than syringe and needle injection (see Figure 9). Initially, both groups of mice lost 18% of their body weight, but the animals receiving the particulate compositions via the PowderJect® device regained their body weight at a quicker rate than the subcutaneously vaccinated mice, indicating that transdermal delivery of particulate immunomodulators to skin is superior to the conventional needle and syringe delivery methodologies.

25

Table 14

30

Protection Against Influenza Challenge

Aluminum (μg)	Influenza Vaccine (μg)	Survived/Total (PowderJect®)	Survived/Total (Syringe/Needle)
none	5	3/8	0/8
none	1	0/8	0/8
100	5	8/8	7/8
100	1	5/7	6/8
-	Naive mice	2/16	

35 Note: Data is the number of animals survived for 14 days versus the total number of animals challenged.

The particulate Alum adjuvant composition was also used as an adjuvant with the crystalline Diphtheria toxoid (dT) vaccine composition and delivered via the PowderJect® needleless syringe delivery system. Mice were vaccinated with 5 μ g or 1 μ g of the crystalline dT vaccine composition either with or without 100 μ g of the particulate Alum adjuvant by transdermal delivery with the PowderJect® device. Control animals were vaccinated subcutaneously with aqueous formulations of the same vaccine/ adjuvant composition using a conventional needle and syringe delivery system. Vaccinations were carried out at weeks 0 and 4. Week 6 sera were collected and pooled from 8 mice, and antibodies to dT were determined by the above-described ELISA techniques.

The results of the ELISA study are reported below in Table 15. As can be seen, serum antibody responses in animals receiving the particulate Alum-adjuvanted dT vaccine composition via the PowderJect® delivery device were significantly higher when compared with control animals receiving the same dose of vaccine without adjuvant. These serum antibody levels were similar to those seen in animals receiving the liquid vaccine/adjuvant composition by conventional means.

Table 15			
IgG Titer in Pooled Week 6 Sera Determined by ELISA			
Aluminum (μ g)	dT (μ g)	Serum IgG Titer (PowderJect®)	Serum IgG Titer (Syringe/Needle)
none	1	8130	570
100	1	58085	142120

The IgG subclass titers to dT were also determined by ELISA, the results of which are reported below in Table 16. As can be seen, transdermal delivery of the particulate Alum-adjuvanted dT vaccine

composition via the PowderJect® device elicited primarily an IgG1 response. A similar IgG subclass distribution was seen following conventional needle and syringe injection. This indicates that the Alum adjuvant promotes a Th2-type immune response to the vaccine upon skin delivery. Thus, particulate Alum adjuvant can be delivered to skin following the methods of the invention and used to control the type of immune response to co-administered vaccines.

10

Table 16					
IgG Subclass Distribution in Pooled Week 6 Sera by ELISA					
Aluminum (μg)	dT (μg)	PowderJect®		Syringe/Needle	
		IgG1	IgG2a	IgG1	IgG2a
none	5	140640	2170	114800	745
100	5	249070	810	359320	1145

2. Vaccination studies with the particulate PCPP adjuvant composition. The particulate PCPP adjuvant composition was used as an adjuvant with the crystalline inactivated influenza vaccine composition and delivered via the PowderJect® needleless syringe delivery system. Mice were vaccinated with 5μg or 1μg of the crystalline inactivated influenza vaccine composition either with or without 100 μg of the particulate PCPP adjuvant composition. Control animals were vaccinated S/C with aqueous formulations of the same vaccine/ adjuvant compositions using a conventional needle and syringe. After three vaccinations were carried out (at weeks 0, 4, and 10.5), week 12 sera were pooled from 8 mice, and antibodies to influenza virus were determined by the above-described ELISA techniques. The injection sites were also visually and manually assessed for signs of toxicity (e.g., granuloma formation).

Granulomas are a common form of local toxic effect seen with the administration of many adjuvants. Subcutaneous injection of the liquid PCPP-adjuvanted influenza vaccine composition resulted in granuloma formation in the subcutaneous tissue at the injection site. In contrast, there were no detectable granulomas following PowderJect® delivery of the particulate composition based on gross hand and visual examination. These data suggest that transdermal delivery of the particulate PCPP adjuvant reduces or even avoids the toxicity commonly associated with PCPP.

In order to assess protection against a subsequent influenza virus challenge, the vaccinated animals were challenged on week 12 by intranasal instillation of 1×10^6 PFU (100X LD₅₀) of a mouse adapted Aichi influenza virus. The challenge was carried out as described above. Survival and body weight loss were recorded daily for 14 days. The survival rates are shown in Table 17 below, and body weights are depicted in Figure 10. As can be seen from Table 17, delivery of the particulate PCPP adjuvant with the crystalline influenza vaccine (at the 5 μ g dose) significantly increased the survival rate when compared with unadjuvanted vaccine. Similar protection was seen with the control (liquid) animals at the same vaccine dose. However, as seen in Figure 10, PowderJect® delivery of the particulate PCPP-adjuvanted influenza vaccine offered better protection against weight loss than subcutaneous injection using a conventional needle and syringe system. In this regard, both groups of mice initially lost about 15% of their body weight, but PowderJect® vaccinated mice regained their body weight at a quicker rate than the subcutaneously vaccinated mice. No significant protection was seen in the animals receiving the 1 μ g

dose of the influenza vaccine (with or without adjuvantation), when delivered in either the particulate or liquid form.

The particulate PCPP adjuvant composition 5 was also used as an adjuvant with the crystalline Diphtheria toxoid (dT) vaccine composition and delivered via the PowderJect® needleless syringe delivery system. Mice were vaccinated with 5 μ g or 1 μ g of the crystalline dT vaccine composition either with 10 or without 100 μ g of the particulate PCPP adjuvant by transdermal delivery with the PowderJect® device. Control animals were vaccinated subcutaneously with 15 aqueous formulations of the same vaccine/ adjuvant composition using a conventional needle and syringe delivery system. Vaccinations were carried out at weeks 0 and 4. Week 6 sera were collected and pooled from 8 mice, and antibodies to dT were determined by the above-described ELISA techniques.

The results of the ELISA study are reported 20 below in Table 18. As can be seen, serum antibody responses in animals receiving the particulate PCPP- adjuvanted dT vaccine composition via the PowderJect® delivery device were significantly higher when compared with control animals receiving the same dose 25 of vaccine without adjuvant. These serum antibody levels were similar to those seen in animals receiving the liquid vaccine/adjuvant composition by conventional means.

30

35

Table 17

Protection Against Influenza Challenge			
PCPP (μ g)	Influenza Vaccine (μ g)	Survived/Total (PowderJect®)	Survived/Total (Syringe/Needle)
none	5	3/8	0/8
none	1	0/8	0/8
100	5	6/8	7/7
100	1	2/8	2/8
-	Naive mice	2/16	

10 Note: Data is the number of animals survived for 14 days versus the total number of animals challenged.

Table 18

IgG Titer in Pooled Week 6 Sera Determined by ELISA			
PCPP (μ g)	dT (μ g)	Serum IgG Titer (PowderJect®)	Serum IgG Titer (Syringe/Needle)
none	1	8130	570
100	1	248550	284415

20 The IgG subclass titers to dT were also determined by ELISA, the results of which are reported below in Table 19. As can be seen, transdermal delivery of the particulate PCPP-adjuvanted dT vaccine composition via the PowderJect® device elicited primarily an IgG1 response. A similar IgG subclass distribution was seen following conventional needle and syringe injection. This indicates that the PCPP adjuvant promotes a Th2-type immune response to the vaccine upon skin delivery. Thus, particulate Alum adjuvant can be delivered to skin following the methods of the invention and used to control the type of immune response to co-administered vaccines.

Table 19

IgG Subclass Distribution in Pooled Week 6 Sera by ELISA					
PCPP (μ g) dT (μ g)	PowderJect®	Syringe/Needle			
		IgG1	IgG2a	IgG1	IgG2a
none	5	140640	2170	114800	745
100	5	308160	3620	585810	3225

3. Vaccination studies with the particulate CpG adjuvant composition. The particulate CpG adjuvant composition was used as an adjuvant with the crystalline inactivated influenza vaccine composition and delivered via the PowderJect® needleless syringe delivery system. Mice were vaccinated with 5 μ g or 1 μ g of the crystalline inactivated influenza vaccine composition either with or without 100 μ g of the particulate CpG adjuvant composition. Control animals were vaccinated S/C with aqueous formulations of the same vaccine/ adjuvant compositions using a conventional needle and syringe. After three vaccinations were carried out (at weeks 0, 4, and 10.5), week 12 sera were pooled from 8 mice, and antibodies to influenza virus were determined by the above-described ELISA techniques.

The results of the ELISA study are reported in Table 20 below. Following delivery of the particulate CpG-adjuvanted influenza vaccine via the PowderJect® device, serum antibody responses were significantly higher when compared with animals receiving the same dose of vaccine without the adjuvant. In addition, serum antibody levels in the animals that received the particulate compositions via the PowderJect® device were significantly higher than serum antibody levels in animals vaccinated with the same vaccine/adjuvant composition (in liquid form) using conventional techniques. Accordingly, the CpG adjuvant can be delivered to the skin in powder form

to enhance the immunogenicity of an influenza vaccine. Delivery in this manner significantly improves the immune enhancement effect provided by the CpG adjuvant.

5

Table 20

Total IgG Titer in Pooled Week 12 Sera Determined by ELISA

CpG (μ g)	Influenza Vaccine (μ g)	Serum IgG Titer (PowderJect®)	Serum IgG Titer (Syringe/Needle)
none	5	6790	1505
none	1	873	804
10 μ g	5	6066	874
10 μ g	1	2862	<100

IgG subclass titers to the influenza virus were also determined by ELISA. The results are reported below in Table 21. As can be seen, PowderJect® delivery of the unadjuvanted particulate influenza vaccine composition elicited primarily an IgG1 response. A similar IgG subclass distribution was seen following conventional needle and syringe injection of the liquid vaccine composition, except that the titer was much lower by this route. These data indicate that influenza vaccine by itself elicits a Th2-type of immunity. PowderJect® delivery of the particulate CpG-adjuvanted influenza vaccine composition elicited primarily IgG2a antibodies, indicating that CpG promotes Th1 response. Thus, skin is a superior site to deliver CpG adjuvant or CpG adjuvanted vaccines.

25

Table 21

IgG Subclass Distribution in Pooled Week 6 Sera by ELISA

30

CpG (μ g)	Influenza Vaccine (μ g)	PowderJect®		Syringe & Needle	
		IgG1	IgG2a	IgG1	IgG2a
none	1	367	<200	<200	<200
10	1	<200	1858	<200	<200
none	5	16340	<200	<200	<200
10	5	714	8711	<200	547

35

In order to assess protection against a subsequent influenza virus challenge, the vaccinated animals were challenged on week 12 by intranasal instillation of 1×10^6 PFU (100X LD₅₀) of a mouse adapted Aichi influenza virus. The viral challenge was carried out as described above. Survival and body weight loss were recorded daily for 14 days. The survival rates are reported in Table 22. Body weight data are depicted in Figures 11A and 11B. As can be seen from Table 22, a 100% survival rate was seen with animals receiving the particulate vaccine compositions (at both the 1 μ g and 5 μ g doses) when adjuvanted with CpG and delivered transdermally via the PowderJect® device. In contrast, subcutaneous injection with the liquid composition did not result in any protection with at the 1 μ g dose. Therefore, PowderJect® delivery of the particulate CpG-adjuvanted influenza vaccine to the skin is more effective than subcutaneous injection.

20

Table 22			
Protection Against Influenza Challenge			
	CpG (μg)	Influenza Vaccine (μg)	Survived/Total (PowderJect®)
25	none	5	3/8
	none	1	0/8
	10	5	7/7
	10	1	8/8
	-	Naive mice	2/16

30

Note: Data is the number of animals survived for 14 days versus the total number of animals challenged.

35

Referring now to Figures 11A and 11B, PowderJect® delivery of the particulate CpG-adjuvanted influenza vaccine composition also offered significantly better protection against weight loss

than subcutaneous injection of the same vaccine composition using conventional needle and syringe delivery. In this regard, there was less than a 10% initial weight loss seen in mice vaccinated with 5 either 1 μ g or 5 μ g of the crystalline influenza vaccine adjuvanted with CpG, and these mice regained their body weight quickly. In contrast, subcutaneous injection offered much less protection. Specifically, there was nearly a 20% initial weight loss in mice 10 subcutaneously injected with the liquid CpG-adjuvanted vaccine (at the 5 μ g dose), and weight recovery took significantly longer. Subcutaneous injection of the liquid CpG-adjuvanted vaccine (at the 1 μ g dose) did not offer any protection. All animals in this control 15 group lost about 25% of their body weight by day 5 and died by day 7. These data suggest that CpG is a much more effective and potent immunomodulator when delivered to the skin using the PowderJect[®] system.

The particulate CpG adjuvant composition was 20 also used as an adjuvant with the crystalline Diphtheria toxoid (dT) vaccine composition and delivered via the PowderJect[®] needleless syringe delivery system. Mice were vaccinated with 5 μ g or 1 μ g 25 of the crystalline dT vaccine composition either with or without 10 μ g of the particulate CpG adjuvant by transdermal delivery with the PowderJect[®] device. Control animals were vaccinated subcutaneously with 30 aqueous formulations of the same vaccine/ adjuvant composition using a conventional needle and syringe delivery system. Vaccinations were carried out at weeks 0 and 4. Week 6 sera were collected and pooled from 8 mice, and antibodies to dT were determined by the above-described ELISA techniques.

The results of the ELISA study are reported 35 below in Table 23. As can be seen, serum antibody responses in animals receiving the particulate CpG-

adjuvanted dT vaccine composition via the PowderJect® delivery device were significantly higher when compared with control animals receiving the same dose of vaccine without adjuvant. Serum antibody levels in the animals receiving the particulate compositions via the PowderJect® delivery device were greater than ten-fold higher than titers in animals vaccinated with the same vaccine/adjuvant composition (in liquid form) via conventional needle and syringe. Thus, delivery of CpG in particulate form enhances the immunogenicity of the co-administered dT vaccine composition.

Table 23

IgG Titer in Pooled Week 6 Sera Determined by ELISA			
CpG (μg)	dT (μg)	Serum IgG Titer (PowderJect®)	Serum IgG Titer (Syringe/Needle)
none	1	8130	570
10	1	614470	33680
none	5	188830	149560
10	5	1483450	116660

IgG subclass titers to the dT antigen were also determined by ELISA. The results are reported below in Table 24. As can be seen, PowderJect® delivery of the unadjuvanted particulate influenza vaccine composition elicited primarily an IgG1 response. A similar IgG subclass distribution was seen following conventional needle and syringe injection of the liquid vaccine composition, except that the titer was much lower by this route. These data indicate that influenza vaccine by itself elicits a Th2- type of immunity. PowderJect® delivery of the particulate CpG-adjuvanted influenza vaccine composition elicited primarily IgG2a antibodies, indicating that CpG promotes Th1 response. A similar IgG subclass distribution was seen following

conventional needle and syringe injection of the same vaccine composition (but in liquid form), except that the titer was ten-fold lower by this route. Thus, skin is a superior site to deliver CpG adjuvant or CpG adjuvanted vaccines.

5
10
15
20

Table 24				
IgG Subclass Distribution in Pooled Week 6 Sera by ELISA				
CpG (μ g)	dT (μ g)	Delivery	IgG Subclass Titer	
			IgG1	IgG2a
none	1	SC	7625	<200
none	1	PJ	605	<200
10	1	SC	297600	14165
10	1	PJ	41850	2350
none	5	SC	140640	2170
none	5	PJ	114800	745
10	5	SC	41050	140200
10	5	PJ	166130	4000

SC = subcutaneous injection, PJ = PowderJect® device.
Vaccinations were given on weeks 0 and 4.

4. Vaccination studies with liquid and particulate MPL adjuvant compositions. In a first study, a liquid MPL adjuvant composition was used as an adjuvant in combination with the crystalline inactivated influenza vaccine composition (delivered via the PowderJect® needleless syringe delivery device). Mice were vaccinated with 5 μ g or 1 μ g of the crystalline inactivated influenza vaccine composition either with or without 50 μ g of the liquid MPL adjuvant composition. When MPL adjuvant was used, the liquid MPL composition was injected intradermally using a 27 gauge needle, and the crystalline vaccine was administered 5 minutes later to the same site using the PowderJect® device. Control animals were

vaccinated S/C with aqueous formulations of the same vaccine/ adjuvant compositions using a conventional needle and syringe. After three vaccinations were carried out (at weeks 0, 4, and 10.5), week 12 sera 5 were pooled from 8 mice, and antibodies to influenza virus were determined by the above-described ELISA techniques.

The results of the ELISA study are reported in Table 25 below. Following skin delivery of the 10 MPL-adjuvanted influenza vaccine, the serum antibody response was significantly higher when compared with animals receiving the same dose of vaccine without the adjuvant. Serum antibody levels in the animals that received the particulate compositions via the 15 PowderJect® device were similar to those from animals that were vaccinated with the same vaccine/adjuvant composition using conventional techniques. Accordingly, the MPL adjuvant can be delivered to the 20 skin in powder form to enhance the immunogenicity of an influenza vaccine.

Table 25

Total IgG Titer in Pooled Week
12 Sera Determined by ELISA

MPL (μ g)	Influenza Vaccine (μ g)	Serum IgG Titer (PowderJect®)	Serum IgG Titer (Syringe/Needle)
none	5	6790	1505
none	1	873	804
50	5	20042	10089
50	1	1158	2490

Note: Vaccinations were given on weeks 0, 4 and 10.5. MPL was injected intradermally using a 27 gauge needle, 5 minutes later vaccine powder was administered to the same site using a PowderJect® device.

IgG subclass titers to the influenza virus 35 were also determined by ELISA. The results are reported below in Table 26. As can be seen,

PowderJect® delivery of the unadjuvanted particulate influenza vaccine composition elicited primarily an IgG1 response. A similar IgG subclass distribution was seen following conventional needle and syringe injection of the liquid vaccine composition. The combination of intradermal MPL adjuvant and PowderJect® delivery of the crystalline influenza vaccine composition elicited both IgG1 and IgG2a antibodies, indicating that skin delivery of MPL induces a balanced Th1/Th2 response to the influenza vaccine. A similar IgG subclass distribution was seen following conventional needle and syringe injection of the liquid vaccine composition.

15

Table 26

20

IgG Subclass Distribution in Pooled Week 6 Sera by ELISA					
MPL (μ g)	Influenza (μ g)	PowderJect®		Syringe & Needle	
none	5	IgG1	IgG2a	IgG1	IgG2a
50	5	18985	1848	9548	3700

25

30

35

In order to assess protection against a subsequent influenza virus challenge, the vaccinated animals were challenged on week 12 by intranasal instillation of 1×10^6 PFU (100X LD₅₀) of a mouse adapted Aichi influenza virus. The challenge was carried out as described above. Survival and body weight loss were recorded daily for 14 days. The survival rates are shown in Table 27 below, and body weight data are depicted in Figure 12. As can be seen from Table 27, 100% survival was seen in animals vaccinated with 5 μ g of the crystalline vaccine composition (delivered via PowderJect® device) adjuvanted with 50 μ g of MPL (delivered via intradermal needle and syringe injection), while only 4 of 6 animals survived in the control (delivery via

conventional needle and syringe) group receiving the same doses of vaccine and adjuvant. Partial protection was seen in animals receiving 1 μ g of the vaccine composition with 50 μ g MPL by both PowderJect® and conventional needle and syringe delivery.

Table 27			
Protection Against Influenza Challenge			
MPL (μ g)	Influenza Vaccine (μ g)	Survived/Total (PowderJect®)	Survived/Total (Syringe/Needle)
none	5	3/8	0/8
none	1	0/8	0/8
50	5	7/7	4/6
50	1	3/8	5/8
-	Naive mice	2/16	

Note: Data is the number of animals survived for 14 days versus the total number of animals after challenge with 10^6 PFUs of virus.

Referring now to Figure 12, PowderJect® delivery of the crystalline influenza vaccine composition (at the 5 μ g dose) coupled with the MPL adjuvant offered significantly better protection against weight loss than subcutaneous injection of the same vaccine/adjuvant combination using conventional needle and syringe delivery. There was maximal weight loss of 10% in animals receiving the 5 μ g vaccine dose (via transdermal PowderJect® delivery) with 50 μ g of the MPL adjuvant, however, these animals quickly regained their weight. In contrast, animals vaccinated with the liquid composition (S/C) lost nearly 20% of their body weight, and their weight recovery progressed at a much slower rate.

In a second study a particulate MPL composition was used as an adjuvant with the crystalline Diphtheria toxoid (dT) vaccine composition and delivered via the PowderJect® needleless syringe

delivery system. Mice were vaccinated with 5 μ g or 1 μ g of the crystalline dT vaccine composition either with or without 50 μ g of the particulate MPL adjuvant composition by transdermal delivery with the 5 PowderJect[®] device. Control animals were vaccinated subcutaneously with aqueous formulations of the same vaccine/ adjuvant composition using a conventional needle and syringe delivery system. Vaccinations were carried out at weeks 0 and 4. Week 6 sera were 10 collected and pooled from 8 mice, and antibodies to dT were determined by the above-described ELISA techniques.

Following transdermal delivery of the particulate MPL-adjuvanted dT vaccine composition, 15 serum antibody responses were marginally higher when compared with control animals receiving the same dose of vaccine without MPL. The level of the serum antibodies in the animals vaccinated via transdermal PowderJect[®] delivery was similar to titers from 20 animals vaccinated with the same vaccine/adjuvant composition by conventional needle and syringe injection.

IgG subclass titers to dT were determined by ELISA. The results are reported in Table 28. As can 25 be seen, PowderJect[®] delivery of unadjuvanted dT vaccine primarily elicited an IgG1 response. A similar IgG subclass distribution was seen following needle and syringe injection. The MPL-adjuvanted dT vaccine composition elicited both IgG1 and IgG2a 30 antibodies when delivered in particulate form from the PowderJect[®] device. A similar IgG subclass distribution was seen following needle and syringe injection, indicating that PowderJect[®] delivery of MPL to the skin can be used to induce a balanced Th1/ Th2- 35 type of immunity.

Table 28

IgG Subclass Distribution in Pooled Week 6 Sera by ELISA				
MPL (μ g)	dT (μ g)	Delivery	IgG Subclass Titer	
			IgG1	IgG2a
none	5	SC	140640	2170
none	5	PJ	114800	745
50	5	SC	258390	10150
50	5	PJ	353300	2925

5 Mouse strain = Balb/C, SC = subcutaneous injection, PJ =
10 PowderJect® device. Vaccinations were given on weeks 0 and 4.

15 Accordingly, novel methods for delivering
vaccines and adjuvant compositions transdermally are
disclosed. Additionally described are novel processed
20 (crystalline) pharmaceutical compositions, and methods
for making and using the same. Although preferred
embodiments of the subject invention have been
described in some detail, it is understood that
obvious variations can be made without departing from
25 the spirit and the scope of the invention as defined
by the appended claims.

25

30

35

We claim:

1. A method for enhancing the immunogenicity of a selected antigen, said method comprising:
 - (a) administering an effective amount of the antigen to a vertebrate subject; and
 - (b) administering an amount of a particulate adjuvant composition sufficient to enhance the immunogenicity of the antigen, wherein the adjuvant is delivered into or across skin or tissue of the vertebrate subject and further wherein said administering is carried out using a transdermal delivery technique.
- 15 2. The method of claim 1, wherein the antigen is in particulate form and is delivered into or across skin or tissue of the vertebrate subject using a transdermal delivery technique.
- 20 3. The method of claim 1, wherein the particulate adjuvant composition is administered using a needleless syringe delivery device.
- 25 4. The method of claim 1, wherein the antigen and adjuvant are present in separate compositions.
- 30 5. The method of claim 1, wherein the antigen and adjuvant are present in the same composition.
- 35 6. The method of claim 1, wherein the antigen and adjuvant are administered to different sites in the vertebrate subject.

7. The method of claim 1, wherein the antigen and adjuvant are administered to the same site in the vertebrate subject.

5 8. The method of claim 1, wherein the antigen is administered prior to the adjuvant composition.

10 9. The method of claim 1, wherein the antigen is administered subsequent to the adjuvant composition.

15 10. The method of claim 1, wherein the antigen is administered concurrently with the adjuvant composition.

11. The method of claim 1, wherein antigen is a viral antigen.

20 12. The method of claim 11, wherein the viral antigen is a viral protein.

13. The method of claim 11, wherein the viral antigen is a viral particle.

25 14. The method of claim 1, wherein the antigen is in a subunit vaccine composition.

30 15. The method of claim 1, wherein the antigen is a bacterial antigen.

16. The method of claim 15, wherein the bacterial antigen is a bacterial protein or polysaccharide.

17. The method of claim 1, wherein the antigen is a live, attenuated organism.

18. The method of claim 17, wherein the
5 attenuated organism is a virus.

19. The method of claim 17, wherein the attenuated organism is a bacterium.

10 20. The method of claim 1, wherein the particulate adjuvant composition is provided in a crystalline form suitable for transdermal delivery.

15 21. The method of claim 1, wherein the adjuvant is a CpG oligonucleotide.

22. The method of claim 21, wherein the CpG oligonucleotide comprises the sequence
TCCATGACGTTCCCTGATGCT (SEQ ID NO:1).

20 23. The method of claim 21, wherein the CpG oligonucleotide comprises the sequence
ATCGACTCTCGAGCGTTCTC (SEQ ID NO:2).

25 24. A method for eliciting an immune response in a vertebrate subject, said method comprising transdermally delivering a particulate vaccine composition into or across skin or tissue of the vertebrate subject, wherein the particulate vaccine composition comprises:

30 (a) an effective amount of a selected antigen; and

(b) an amount of an adjuvant sufficient to enhance the immunogenicity of the antigen.

35

25. The method of claim 24, wherein the particulate vaccine composition is administered using a needleless syringe delivery device.

5 26. The method of claim 24, wherein the antigen is a viral antigen.

27. The method of claim 26, wherein the viral antigen is a viral protein.

10 28. The method of claim 26, wherein the viral antigen is a viral particle.

15 29. The method of claim 24, wherein the vaccine composition is a subunit vaccine composition.

30. The method of claim 24, wherein the antigen is a bacterial antigen.

20 31. The method of claim 30, wherein the bacterial antigen is a bacterial protein or polysaccharide.

25 32. The method of claim 24, wherein the antigen is a live, attenuated organism.

33. The method of claim 32, wherein the attenuated organism is a virus.

30 34. The method of claim 32, wherein the attenuated organism is a bacterium.

35 35. The method of claim 24, wherein the particulate vaccine composition is provided in a crystalline form suitable for transdermal delivery.

36. The method of claim 24, wherein the adjuvant is a CpG oligonucleotide.

37. The method of claim 36, wherein the CpG oligonucleotide comprises the sequence
5 TCCATGACGTTCTGATGCT (SEQ ID NO:1).

38. The method of claim 36, wherein the CpG oligonucleotide comprises the sequence
10 ATCGACTCTCGAGCGTTCTC (SEQ ID NO:2).

39. A particulate adjuvant composition suitable for delivery into or across skin or tissue of a vertebrate subject using a transdermal delivery
15 technique.

40. Use of an adjuvant in the manufacture of a particulate composition for transdermal delivery into or across skin or tissue of a vertebrate subject.
20

41. The use according to claim 40, wherein the particulate composition comprises a selected antigen and the adjuvant enhances the immunogenicity of the antigen.
25

42. The use according to claim 40, wherein the particulate composition is delivered into or across skin or tissue of the vertebrate subject using a needleless syringe delivery device.
30

43. The use according to claim 40, wherein antigen is a viral antigen.
35

44. The use according to claim 43, wherein the viral antigen is a viral protein.

45. The use according to claim 43, wherein the viral antigen is a viral particle.

46. The use according to claim 40, wherein 5 the antigen is in a subunit vaccine composition.

47. The use according to claim 40, wherein the antigen is a bacterial antigen.

10 48. The use according to claim 47, wherein the bacterial antigen is a bacterial protein or polysaccharide.

15 49. The use according to claim 40, wherein the antigen is a live, attenuated organism.

50. The use according to claim 49, wherein the attenuated organism is a virus.

20 51. The use according to claim 49, wherein the attenuated organism is a bacterium.

25 52. The use according to claim 40, wherein the particulate composition is provided in a crystalline form suitable for transdermal delivery.

53. The use according to claim 40, wherein the adjuvant is a CpG oligonucleotide.

30 54. The use according to claim 53, wherein the CpG oligonucleotide comprises the sequence TCCATGACGTTCTGATGCT (SEQ ID NO:1).

35 55. The use according to claim 53, wherein the CpG oligonucleotide comprises the sequence ATCGACTCTCGAGCGTTCTC (SEQ ID NO:2).

56. A method of eliciting a physiological effect in a vertebrate subject comprising administering an amount of the particulate adjuvant composition of claim 39 into or across skin or tissue 5 of the vertebrate subject sufficient to bring about the physiological effect.

57. A method for forming a crystalline pharmaceutical composition, said method comprising:

10 (a) combining a liquid pharmaceutical formulation with a suitable pharmaceutical grade sugar to provide a composition;

15 (b) allowing the composition to dry under suitable evaporative conditions which favor crystal formation, thereby obtaining a crystalline composition having enhanced density characteristics; and

(c) collecting the crystalline composition.

58. The method of claim 57, wherein the 20 pharmaceutical composition is a vaccine composition.

59. A crystalline pharmaceutical composition suitable for delivery into or across skin or tissue of a vertebrate subject.

25

60. The composition of claim 59, wherein said composition is a vaccine composition.

30

61. The composition of claim 60, wherein said composition comprises an antigen and an excipient in an amount sufficient to enhance the density of the crystalline pharmaceutical composition.

35

62. The composition of claim 61, wherein the antigen is a viral antigen.

63. The composition of claim 61, wherein the antigen is a bacterial antigen.

64. A method for treating a subject, said method comprising delivering the crystalline pharmaceutical composition of claim 59 into or across skin or tissue of said subject, wherein the crystalline composition is delivered in an amount sufficient to bring about a prophylactic or therapeutic effect in the subject.

65. The method of claim 64, wherein the pharmaceutical composition is a vaccine composition comprising an antigen of interest.

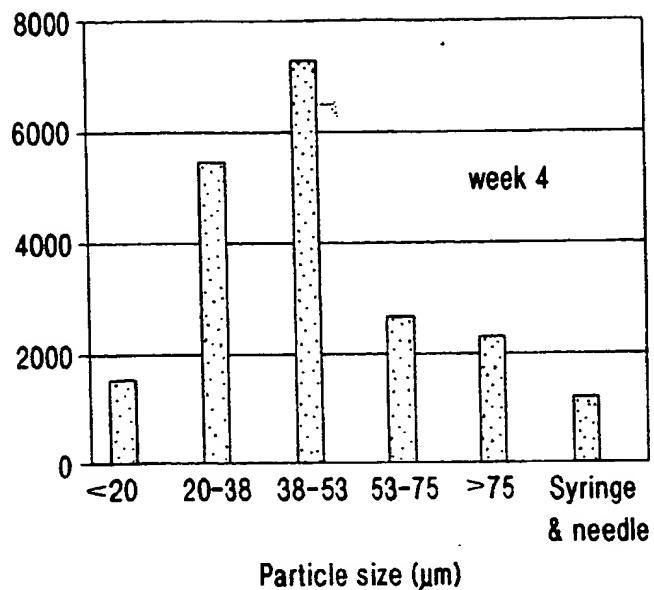
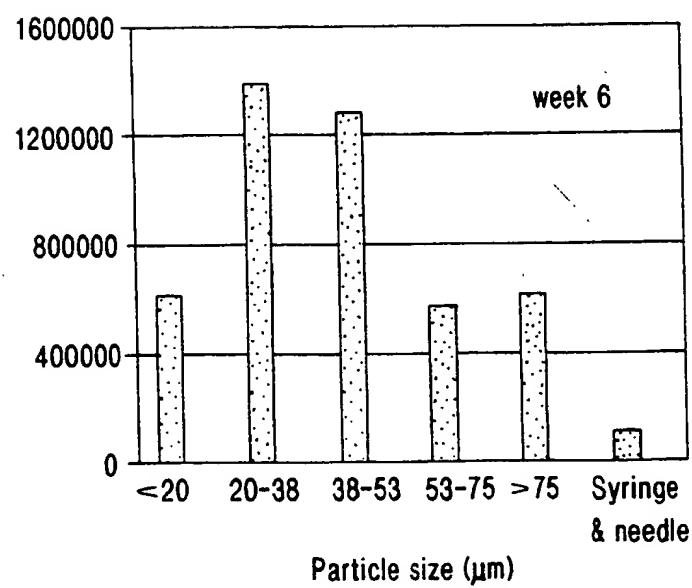
66. The method of claim 65, wherein the vaccine composition is a subunit vaccine composition.

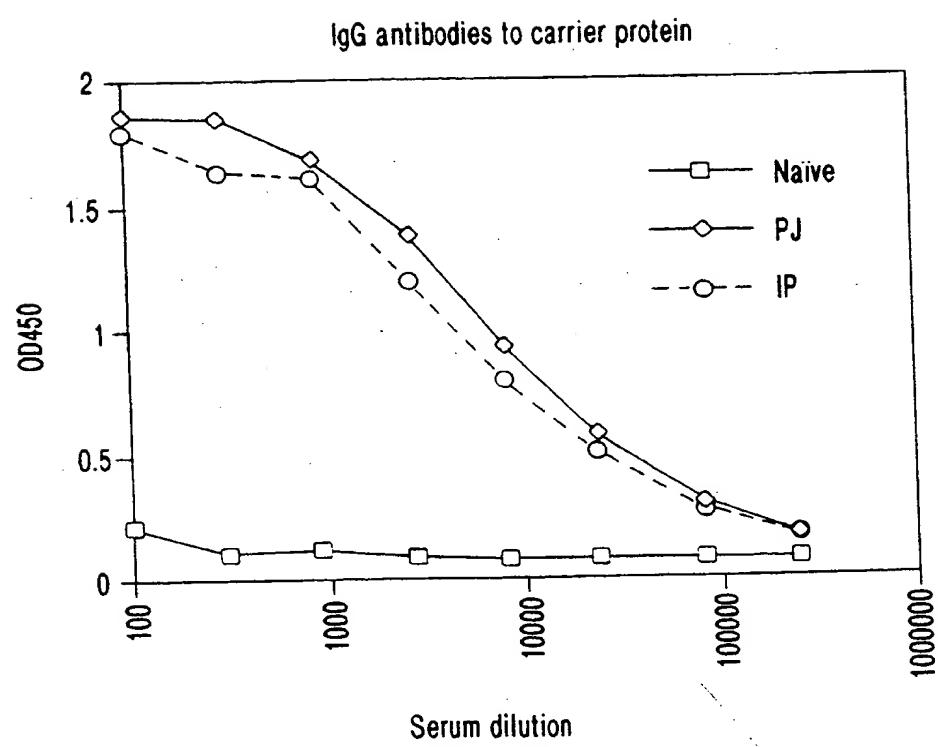
67. The method of claim 65, wherein the vaccine composition comprises a viral antigen.

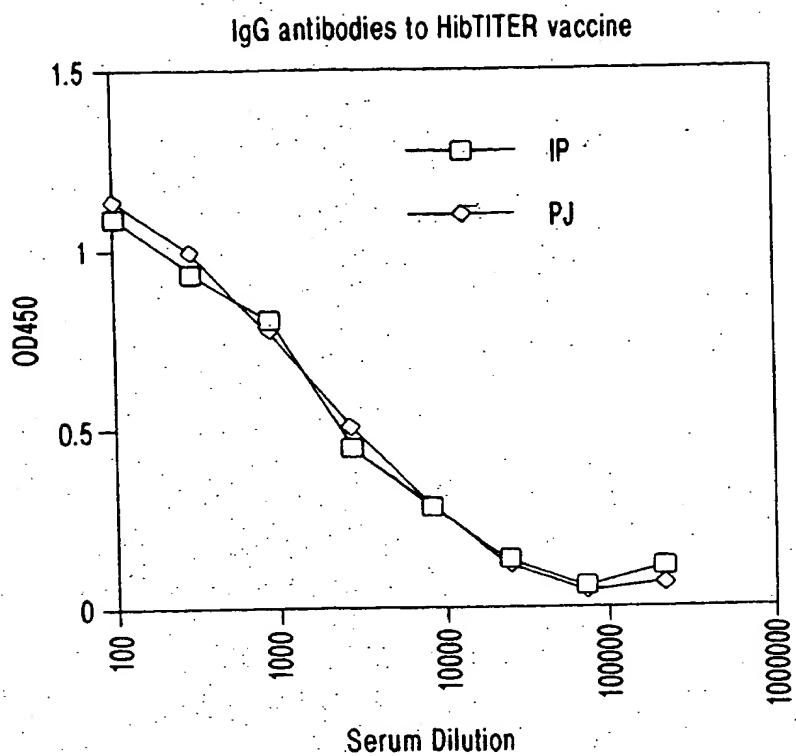
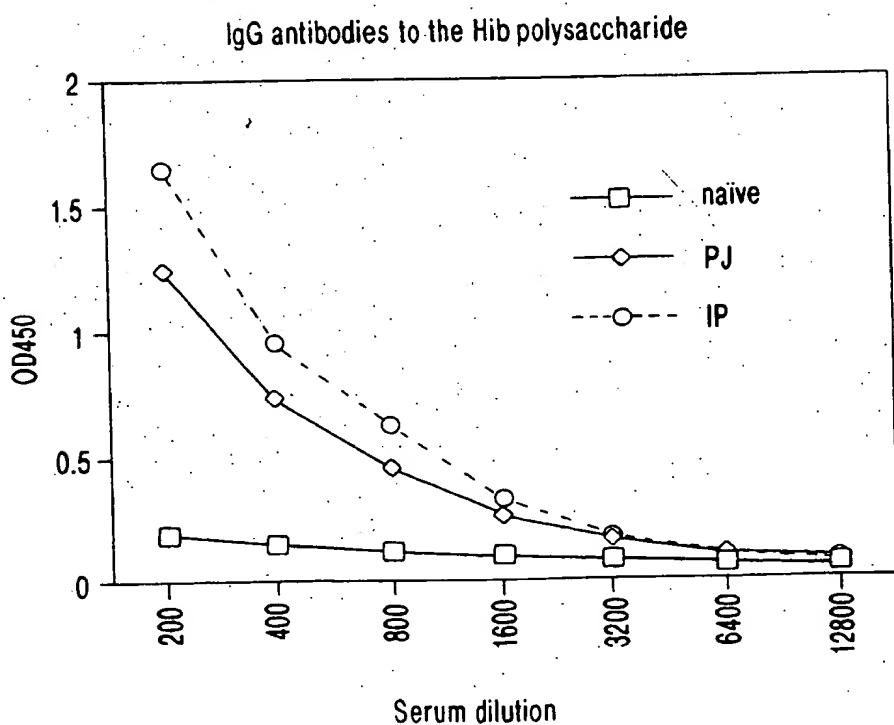
68. The method of claim 65, wherein the vaccine composition comprises a bacterial antigen.

69. The method of claim 64, wherein the crystalline composition is delivered to the subject using a needleless syringe.

70. Use of a pharmaceutical agent in the manufacture of a crystalline composition for transdermal delivery into or across skin or tissue of a vertebrate subject.

**FIG. 1A****FIG. 1B**

**FIG. 2**

**FIG. 3**

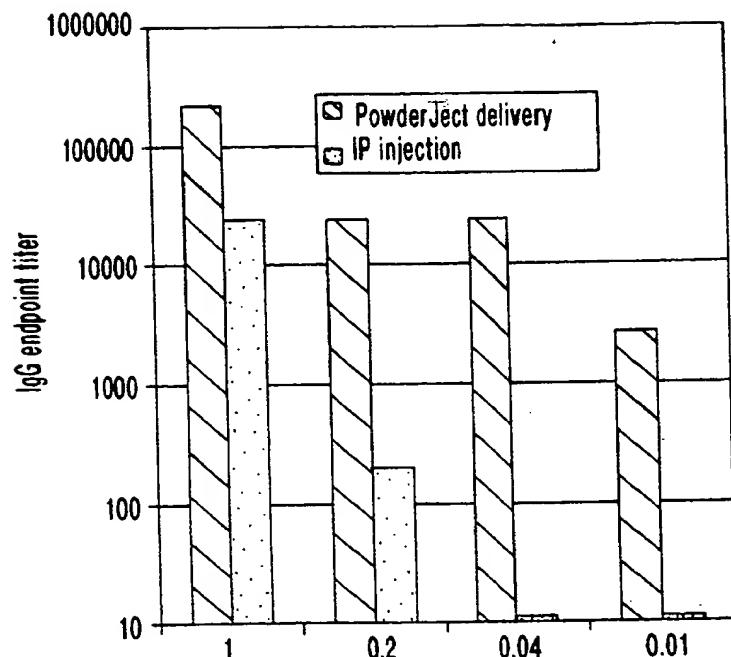


FIG. 5

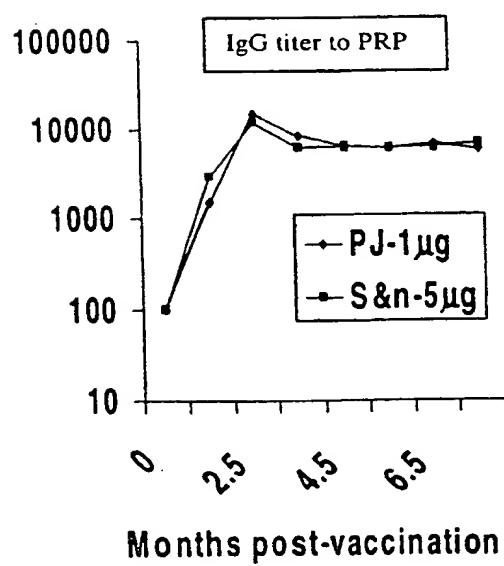


FIG. 6A

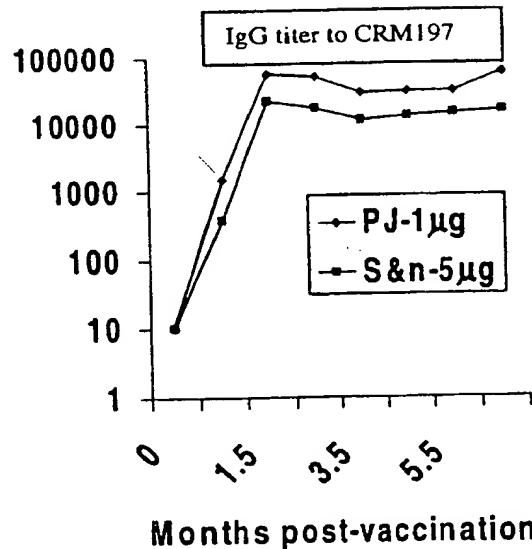


FIG. 6B

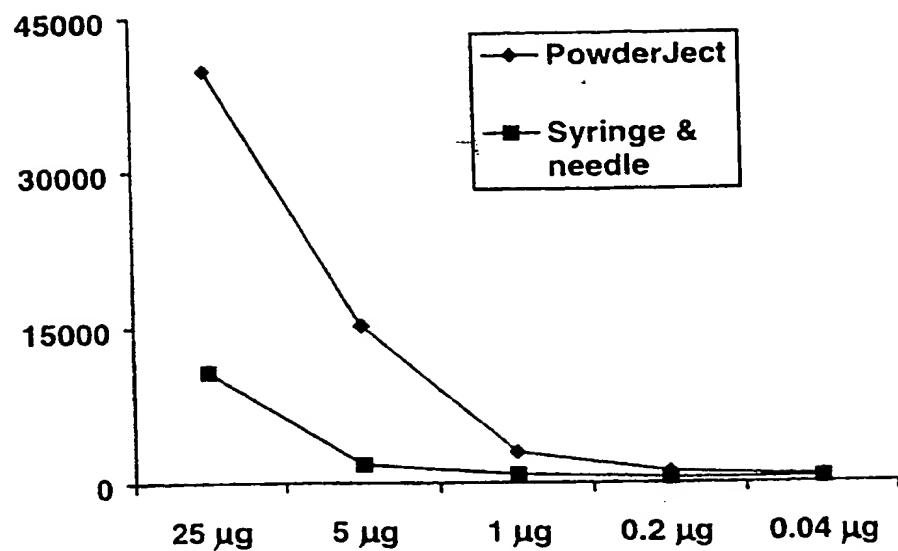


FIG. 7

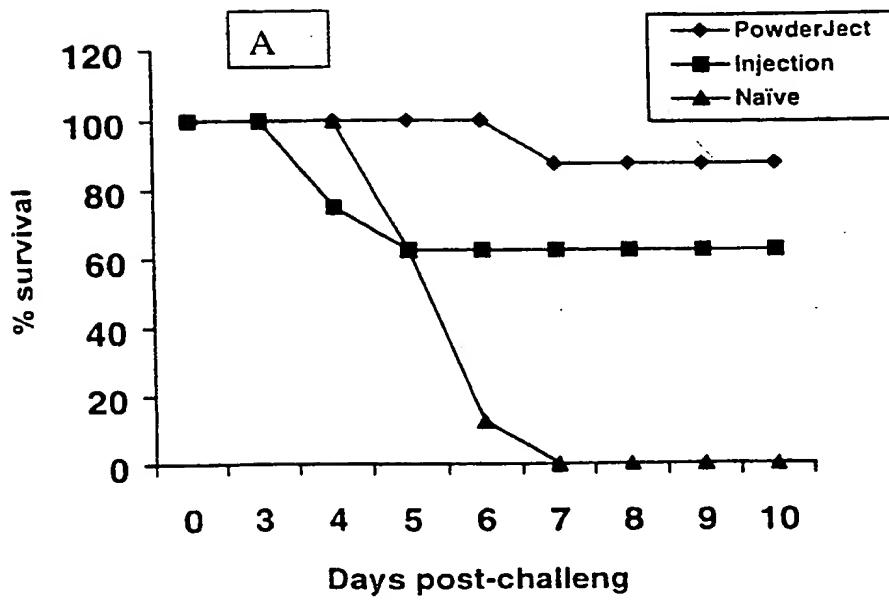


FIG. 8A

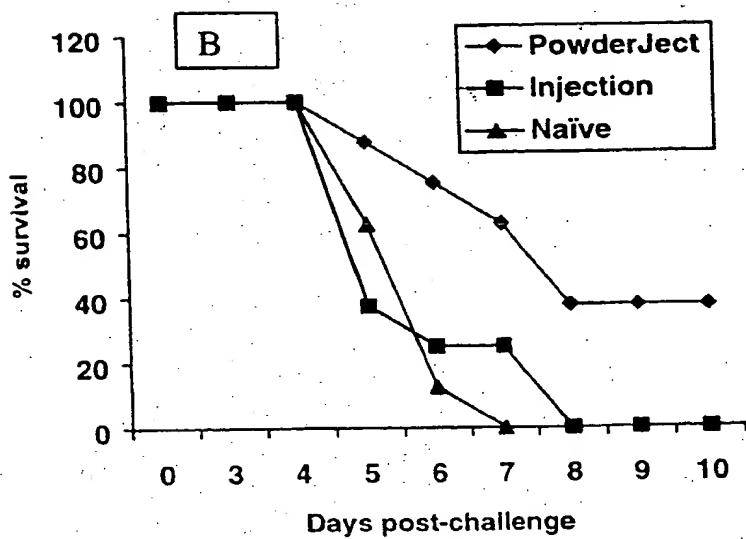


FIG. 8B

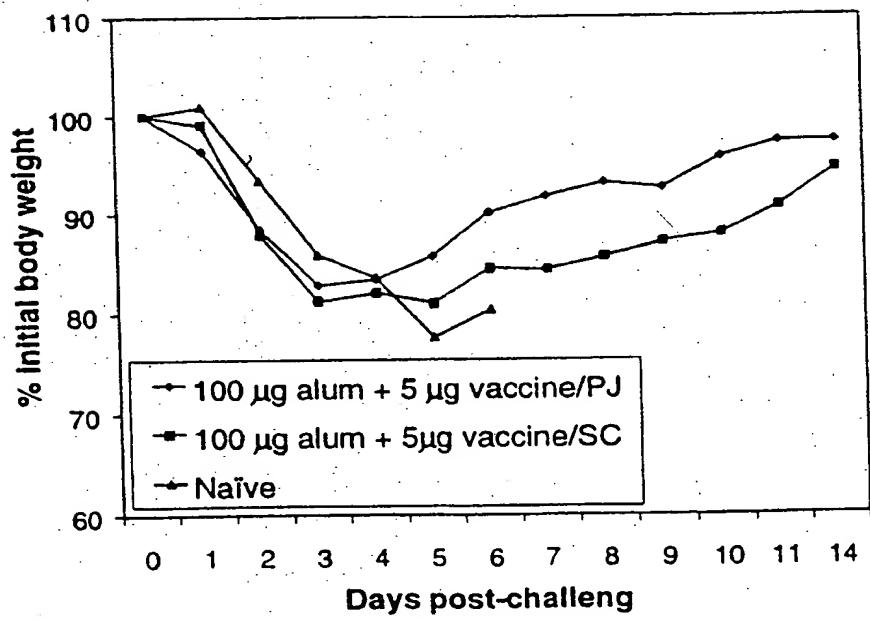


FIG. 9

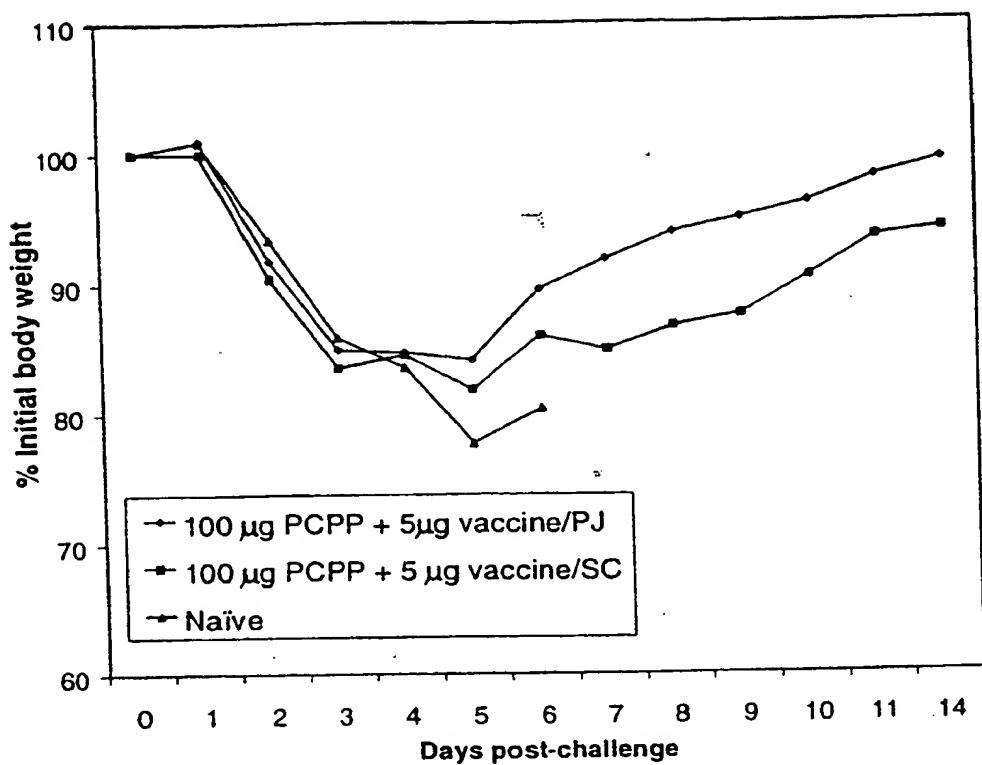
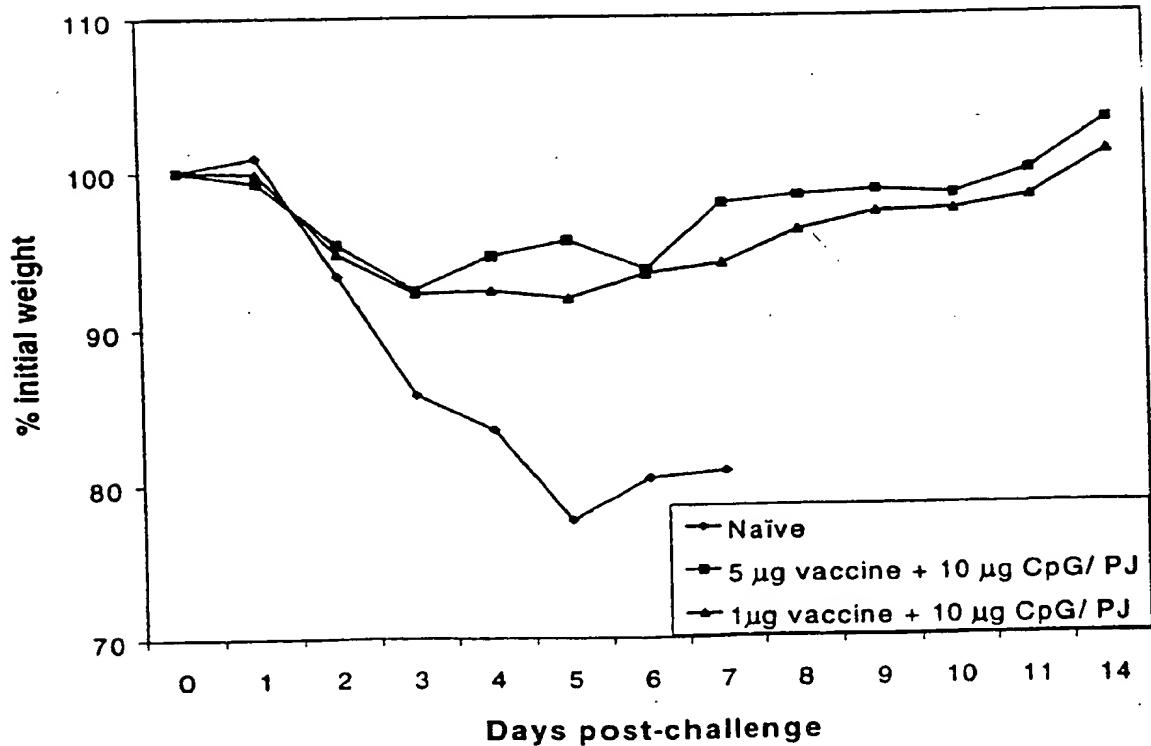


FIG. 10



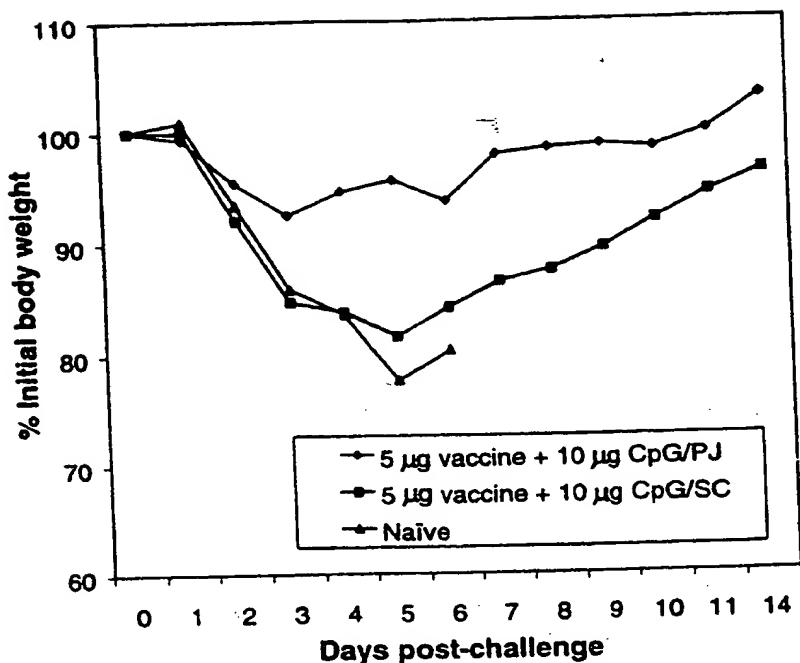


FIG. 11B

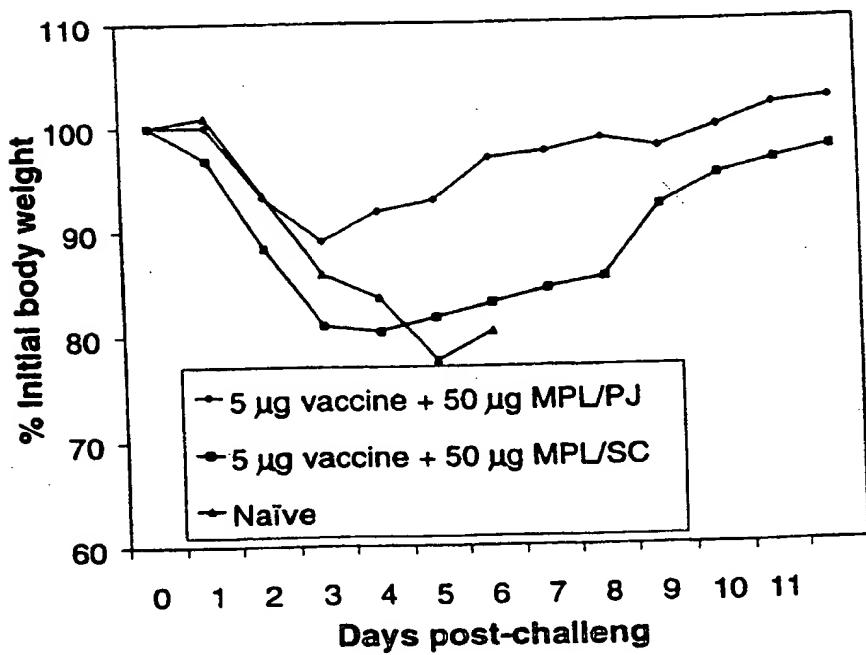


FIG. 12

INTERNATIONAL SEARCH REPORT

International Application

PCT/US 98/25563

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K39/39 A61K9/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>WO 90 01949 A (UNIV AUSTRALIAN) 8 March 1990</p> <p>see page 1, line 32 - page 3, line 31 see page 5, line 6 - line 27 see examples 1,4</p> <p>---</p> <p>-/-</p>	<p>1,2, 4-20,24, 26-35, 39-41, 43-52, 56, 59-68,70</p>

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"Z" document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

21 April 1999

06/05/1999

Name and mailing address of the ISA
European Patent Office, P B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. +31 70 340 2030, Tx. 31 651 300 01

Authorized officer

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/25563

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 091 188 A (HAYNES DUNCAN H) 25 February 1992 see claim 1; example 16 ---	1,2, 4-20,24, 26-35, 39-41, 43-52, 56, 59-68,70
X	WO 94 09819 A (DUNCAN JACQUELINE D) 11 May 1994 see page 5, line 3 - page 6, line 19 see page 10, line 18 - line 22; example 5 see page 22, line 5 - line 14 ---	1,2, 4-20,24, 26-35, 39-41, 43-52, 56-68,70
X	WO 97 40163 A (COLPAN METIN ;SCHORR JOACHIM (DE); BAKER HENRY J (US); SMITH BRUCE) 30 October 1997 see page 12 - page 14 see claims 17-22 ---	1-22, 24-37, 39-54, 56,59-70
X	EP 0 390 435 A (TAKEDA CHEMICAL INDUSTRIES LTD) 3 October 1990 see page 3, line 18-30; examples 1,2,4,5 ---	57
X	"REMINGTON'S PHARMACEUTICAL SCIENCES" 1980 . MARK PUBLISHING COMPANY , PENNSYLVANIA XP002100472 see page 1535 "spray drying" and pages 1483-1484, "freeze drying", especially page 1484 "factors affecting formulation" ---	57
X	US 4 629 782 A (CHAN TAI W ET AL) 16 December 1986 see the whole document ---	39,40, 42,52, 59,60,70
A	SARPHIE D F ET AL: "BIOAVAILABILITY FOLLOWING TRANSDERMAL POWDERED DELIVERY (TPD) OF RADIOLABELED INULIN TO HAIRLESS GUINEA PIGS" JOURNAL OF CONTROLLED RELEASE, vol. 47, no. 1, 7 July 1997, pages 61-69, XP000685875 see the whole document ---	1-70

-/-

INTERNATIONAL SEARCH REPORT

International Application

PCT/US 98/25563

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 94 24263 A (SARPHIE DAVID FRANCIS ; BELLHOUSE BRIAN JOHN (GB); GREENFORD JOHN C) 27 October 1994 see the whole document, especially page 3, lines 13-36 see the whole document -----	1-70

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 98/25563

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

- 1. Claims Nos.: 1-38, 56, 64-69 because they relate to subject matter not required to be searched by this Authority, namely:
See FURTHER INFORMATION SHEET PCT/ISA/210
- 2. Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
- 3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

- 1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
- 2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
- 3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
- 4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 98/25563

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Although claims 1-38, 56 and 64-69 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the composition.

Claims Nos.: 1-38 56 64-69

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/25563

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 9001949	A 08-03-1990	AU 4187689	A	23-03-1990
		CA 1337047	A	19-09-1995
		DE 68922109	D	11-05-1995
		DE 68922109	T	12-10-1995
		EP 0431023	A	12-06-1991
		US 5476844	A	19-12-1995
US 5091188	A 25-02-1992	AU 7852891	A	11-11-1991
		CA 2078990	A	27-10-1991
		EP 0533690	A	31-03-1993
		IN 173056	A	05-02-1994
		MX 25532	A	01-10-1993
		RU 2100030	C	27-12-1997
		WO 9116068	A	31-10-1991
		US RE35338	E	24-09-1996
		US 5091187	A	25-02-1992
		US 5246707	A	21-09-1993
WO 9409819	A 11-05-1994	AU 5547594	A	24-05-1994
WO 9740163	A 30-10-1997	AU 2699497	A	12-11-1997
		CA 2252375	A	30-10-1997
EP 0390435	A 03-10-1990	AT 99545	T	15-01-1994
		DE 69005679	D	17-02-1994
		DE 69005679	T	23-06-1994
		DK 390435	T	21-03-1994
		ES 2062341	T	16-12-1994
		JP 3007230	A	14-01-1991
		US 5064829	A	12-11-1991
US 4629782	A 16-12-1986	NONE		
WO 9424263	A 27-10-1994	AT 148497	T	15-02-1997
		AU 674742	B	09-01-1997
		AU 6435194	A	08-11-1994
		BG 61993	B	30-12-1998
		BG 100047	A	30-04-1996
		BR 9406455	A	02-01-1996
		CA 2159452	A	27-10-1994
		CN 1120852	A	17-04-1996
		CZ 9502608	A	15-05-1996
		DE 69401651	D	13-03-1997
		DE 69401651	T	15-05-1997
		DK 693119	T	28-07-1997
		EP 0693119	A	24-01-1996
		EP 0734737	A	02-10-1996
		ES 2098131	T	16-04-1997
		FI 954788	A	06-10-1995
		GR 3022939	T	30-06-1997
		HK 1000351	A	06-03-1998
		HU 73516	A	28-08-1996
		JP 8509604	T	15-10-1996
		LV 11833	A	20-08-1997
		LV 11833	B	20-12-1997
		NO 953994	A	06-10-1995
		NZ 263606	A	22-08-1997
		PI 311005	A	22-01-1996

INTERNATIONAL SEARCH REPORT

In: International Application

PCT/US 98/25563

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9424263 A		SG 48696 A	18-05-1998
		SI 693119 T	31-10-1997
		SK 124895 A	08-01-1997
		US 5630796 A	20-05-1997
		ZA 9402442 A	10-04-1995

THIS PAGE BLANK (USPTO)